
Subpart K--Quality System for Nonwaived Testing

§493.1200 Introduction.

- (a) Each laboratory that performs nonwaived testing must establish and maintain written policies and procedures that implement and monitor a quality systems for all phases of the total testing process (that is, preanalytic, analytic, and postanalytic) as well as general laboratory systems.
- (b) The laboratory's quality systems must include a quality assessment component that ensures continuous improvement of the laboratory's performance and services through ongoing monitoring that identifies, evaluates and resolves problems.
- (c) The various component of the laboratory's quality system are used to meet the requirements in this part and must be appropriate for the specialties and subspecialties of testing the laboratory performs, services it offers, and clients it serves.

D5002

§493.1201 Condition: Bacteriology.

If the laboratory provides services in the subspecialty of Bacteriology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1261, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1201

Tests or procedures to detect an antigen are categorized in this subspecialty where the antigen is detected or identified. For example, tests or procedures for identifying Group A Streptococcus are categorized in Bacteriology.

D5004

§493.1202 Condition: Mycobacteriology.

If the laboratory provides services in the subspecialty of Mycobacteriology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1262, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1202

Tests or procedures to detect an antigen are categorized in the subspecialty where the antigen is detected or identified. For example, the procedures to identify Mycobacteria are categorized in Mycobacteriology.

D5006

§493.1203 Condition: Mycology.

If the laboratory provides services in the subspecialty of Mycology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1263, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1203

Tests or procedures to detect an antigen are categorized in the subspecialty where the antigen is detected or identified. For example, tests for the identification of fungi are categorized in Mycology.

D5008

§493.1204 Condition: Parasitology.

If the laboratory provides services in the subspecialty of Parasitology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1264, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1204

Tests or procedures to identify an antigen are categorized in the subspecialty where the antigen is detected or identified. For example, procedures to identify a parasite are categorized in the subspecialty of Parasitology; however, procedures to detect or identify an antibody to the parasite are categorized in the subspecialty of General Immunology.

D5010

§493.1205 Condition: Virology.

If the laboratory provides services in the subspecialty of Virology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1265, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1205

Tests or procedures to identify the virus (antigen) are categorized in the subspecialty when the antigen is detected or identified. For example, tests or procedures to detect herpes are categorized in the subspecialty of Virology. Tests or procedures to detect antibodies to Herpes are categorized in the subspecialty of General Immunology.

D5012

§493.1207 Condition: Syphilis serology.

If the laboratory provides services in the subspecialty of Syphilis serology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1207

Quality control requirements for Syphilis Serology are found in §493.1256. Use D5441 through D5485 as appropriate.

D5014

§493.1208 Condition: General immunology.

If the laboratory provides services in the subspecialty of General immunology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1208

Tests or procedures to detect or identify antibodies to a bacteria, virus, parasite, etc., are categorized under the subspecialty of General Immunology. Quality control requirements for General Immunology are found at §493.1256.

D5016

§493.1210 Condition: Routine chemistry.

If the laboratory provides services in the subspecialty of Routine chemistry, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1267, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1210

Quality control requirements for Routine Chemistry are found in §§493.1256 and 493.1267. Use D5441 through D5485 and D5535 through D5539, as appropriate.

D5018

§493.1211 Condition: Urinalysis.

If the laboratory provides services in the subspecialty of Urinalysis, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1211

Quality control requirements for the subspecialty of Urinalysis are found in §493.1256. Use D5441 through D5485, as appropriate.

D5020

§493.1212 Condition: Endocrinology.

If the laboratory provides services in the subspecialty of Endocrinology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1212

Quality control requirements for the subspecialty of Endocrinology are found in §493.1256. Use D5441 through D5485, as appropriate.

D5022

§493.1213 Condition: Toxicology.

If the laboratory provides services in the subspecialty of Toxicology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1213

Quality control requirements for the subspecialty of Toxicology are found in §493.1256. Use D5441 through D5485, as appropriate.

D5024

§493.1215 Condition: Hematology.

If the laboratory provides services in the specialty of Hematology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1269, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1215

Quality control requirements for the subspecialty of automated Hematology are found in §493.1256. Use D5441 through D5485, as appropriate.

D5026

§493.1217 Condition: Immunohematology.

If the laboratory provides services in the specialty of Immunohematology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1271, and §§493.1281 through 493.1299.

D5028

§493.1219 Condition: Histopathology.

If the laboratory provides services in the subspecialty of Histopathology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1273, and §§493.1281 through 493.1299.

D5030

§493.1220 Condition: Oral pathology.

If the laboratory provides services in the subspecialty of Oral pathology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, and §§493.1281 through 493.1299.

D5032

§493.1221 Condition: Cytology.

If the laboratory provides services in the subspecialty of Cytology, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1274, and §§493.1281 through 493.1299.

D5034

§493.1225 Condition: Clinical cytogenetics.

If the laboratory provides services in the specialty of Clinical cytogenetics, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1276, and §§493.1281 through 493.1299.

D5040

§493.1226 Condition: Radiobioassay.

If the laboratory provides services in the specialty of Radiobioassay, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, and §§493.1281 through 493.1299.

Interpretative Guidelines §493.1226

Quality control requirements for the subspecialty of Radiobioassay are found in §493.1256. Use D5441 through D5485, as appropriate.

D5042

§493.1227 Condition: Histocompatibility.

If the laboratory provides services in the specialty of Histocompatibility, the laboratory must meet the requirements specified in §§493.1230 through 493.1256, §493.1278, and §§493.1281 through 493.1299.

D5200

General Laboratory Systems

§493.1230 Condition: General laboratory systems.

Each laboratory that performs nonwaived testing must meet the applicable general laboratory systems requirements in §§493.1231 through 493.1236, unless HHS approves a procedure, specified in Appendix C of the State Operations Manual #7, that provides equivalent quality testing. The laboratory must monitor and evaluate the overall quality of the general laboratory systems and correct identified problems specified in §493.1239 for each specialty and subspecialty of testing performed.

Interpretative Guidelines §493.1230

Significant deficiencies cited under this condition may indicate deficiencies under personnel responsibilities. Use D5200 when significant deficiencies are identified and have the potential to, or adversely affect patient testing, are systemic and pervasive throughout the laboratory, and are not limited to any one specialty or subspecialty.

The requirements in this section address those general operational functions that are not specific to any one specialty or subspecialty.

D5201

§493.1231 Standard: Confidentiality of patient information.

The laboratory must ensure confidentiality of patient information throughout all phases of the total testing process that are under the laboratory's control.

Probes §493.1231

How does the laboratory "control" visitor access to the laboratory areas where patient information may be easily viewed (e.g., computer terminals, facsimile machines, worksheets)?

Are there safeguards in place to ensure confidentiality of patient information and test reports? For example, are unauthorized users prohibited from gaining entry?

How does the laboratory ensure its record storage system(s) is secure?

D5203

§493.1232 Standard: Specimen identification and integrity.

The laboratory must establish and follow written policies and procedures that ensure positive identification and optimum integrity of a patient's specimen from the time of collection or receipt of the specimen through completion of testing and reporting of results.

Interpretative Guidelines §493.1232

The regulation provides laboratories the flexibility to establish a system that ensures positive patient identification through specimen collection, labeling, accessioning, processing, (e.g., aliquotting), storage, testing, and reporting of results. Review the laboratory's system (policy and practices) for ensuring positive patient identification from specimen collection through reporting of results.

Optimum integrity of a patient's specimen should be determined according to the test methodology utilized by the laboratory. Review manufacturer's instructions for performance of each test method to ensure the specimen is appropriate for the test system, is stored properly (e.g., maintained at room temperature, kept on ice, separated and frozen or refrigerated), and analyzed within the limitations of the test methodology.

The laboratory must have a procedure to ensure that special handling of specimens is maintained throughout the testing process when necessary, (e.g., GC cultures and GC/Chlamydia probes, blood gas specimens, and DNA probes.)

Probes §493.1232

How does the laboratory ensure positive identification of patient specimens through all phases of testing, especially when similar patient identification information (e.g., address, sex, names, timed specimens, and birth dates) exists?

How does the laboratory assure that special handling of specimens (when specified by the testing laboratory) is maintained throughout the testing process?

Does the laboratory process patient specimens using separate (distinct) or unique identifiers in order to avoid mislabeling, specimen mix-ups, incorrect test request entry, and incorrect reporting of results?

D5205

§493.1233 Standard: Complaint investigations.

The laboratory must have a system in place to ensure that it documents all complaints and problems reported to the laboratory. The laboratory must conduct investigations of complaints, when appropriate.

Interpretative Guidelines §493.1233

Verify that the laboratory documents all complaints and problems reported to the laboratory, and has a mechanism to determine which complaints require investigation.

For Immunohematology complaints related to transfusion reaction investigation, use D3130 or D5559, as appropriate.

Probes §493.1233

What mechanism does the laboratory have that allows individuals to report complaints or problems to the laboratory?

Does the laboratory have a mechanism to refer complaints or problems to its reference laboratory(s), or other offices or agencies, when appropriate? Does the laboratory document this activity?

D5207

§493.1234 Standard: Communications.

The laboratory must have a system in place to identify and document problems that occur as a result of a breakdown in communication between the laboratory and an authorized person who orders or receives test results.

Interpretative Guidelines §493.1234

Communication may begin with the test request of information requested concerning patient specimens. If the laboratory does not receive the appropriate specimen or patient information needed to perform the tests, the laboratory should assess the information concerning patient preparation and specimen handling requirements provided to the authorized individuals.

The test report form should be easily understood and accurately portray patient test results and other information necessary for interpreting test results (e.g., failure to identify peak and trough).

D5209

§493.1235 Standard: Personnel competency assessment policies.

As specified in the personnel requirements in subpart M, the laboratory must establish and follow written policies and procedures to assess employee and, if applicable, consultant competency.

Interpretative Guidelines §493.1235

Refer to §§493.1413(b)(8) and 493.1451(b)(8) for specific testing personnel competency requirements and refer to §493.1407(e)(12) and §493.1445(e)(13) for establishing policies to monitor each individual's competency and identify remedial training or continuing education needs. Cite deficiencies at this location when the laboratory has developed but is not following personnel competency policies and procedures for technical and clinical consultants, technical supervisors and other laboratory staff.

For microscopic urinalysis, the laboratory must have a system that ensures all personnel report microscopic morphologic data on patient samples in a similar fashion. For initial accuracy, as well as consistency in serial samples from the same patient, the laboratory should assess its staff for consistency with respect to morphologic classification.

Suggested methods include:

- Circulation of preserved urine sediments with leukocytes, erythrocytes, casts, bacteria, yeast, etc.
- Use of multi-headed microscopes
- Use of urine sediment photomicrographs

Probes §493.1235

How does the laboratory evaluate the competency of its employees?

If the laboratory uses non-testing personnel to perform preanalytic functions how does it ensure their competency?

If a laboratory utilizes a consultant, how does the laboratory determine if the consultant is competent? Does the laboratory have a policy/procedure to determine consultant competency? Use D6030 or D6103.

How does the laboratory evaluate personnel for consistency in slide review, (e.g., ANA, manual differential, urine sediment)?

D5211

§493.1236 Standard: Evaluation of proficiency testing performance.

(a) The laboratory must review and evaluate the results obtained on proficiency testing performed as specified in subpart H of this part.

Probes §493.1236(a)

Is there evidence of review and evaluation of the laboratory's proficiency testing (PT) results?

§493.1236 Standard: Evaluation of proficiency testing performance.

(b) The laboratory must verify the accuracy of the following:

D5213

§493.1236 Standard: Evaluation of proficiency testing performance.

(b)(1) Any analyte or subspecialty without analytes listed in subpart I of this part that is not evaluated or scored by a CMS-approved proficiency testing program.

Interpretative Guideline §493.1236(b)(1)

An analyte may not be evaluated or scored by the PT program if there are less than 10 participants in a particular peer group.

D5215

§493.1236 Standard: Evaluation of proficiency testing performance.

(b)(2) Any analyte, specialty or subspecialty assigned a proficiency testing score that does not reflect laboratory test performance (that is, when the proficiency testing program does not obtain the agreement required for scoring as specified in subpart I of this part, or the laboratory receives a zero score for nonparticipation, or late return or results).

Interpretative Guidelines §493.1236(b)(2)

The laboratory must have a mechanism for routine review of its proficiency testing results that are evaluated by its PT providers. This includes a review of its actual PT results against the PT provider's participant summary results for the particular PT event and when any of the following occur:

- The PT program assigned an artificial score of 100% (e.g., results not evaluated or scored);
- A zero score for nonparticipation; if the laboratory did not test the specimen, it must document what other means were used to assess the accuracy of the test for the PT event that was missed; or
- The PT provider notifies the laboratory that its results were not evaluated (given a score of "0") due to missing the return deadline.

Probes §493.1236(b)(2)

Has the laboratory reviewed its test menu to determine if it tests any analyte(s) that are not listed in subpart I?

§493.1236 Standard: Evaluation of proficiency testing performance.

(c) At least twice annually, the laboratory must verify the accuracy of the following:

D5217

§493.1236 Standard: Evaluation of proficiency testing performance.

(c)(1) Any test or procedure it performs that is not included in subpart I of this part.

Interpretative Guidelines §493.1236(c)(1)

Refer to subpart I, Proficiency Testing Programs for Nonwaived Testing. Subpart I includes those specialties, subspecialties, and analytes that are considered regulated tests. For those tests not listed in subpart I (not regulated), the laboratory must verify the accuracy of the test or procedure twice annually.

D5219

§493.1236 Standard: Evaluation of proficiency testing performance.

(c)(2) Any test or procedure listed in subpart I of this part for which compatible proficiency testing samples are not offered by a CMS-approved proficiency testing program.

Interpretative Guidelines §493.1236(c)(2)

Laboratory tests or procedures that are not compatible may include new or emerging technologies for which PT is not yet available.

Probes §493.1236(c)(2)

How does the laboratory verify accuracy of tests not included under subpart I or tests for which compatible PT samples are not available (e.g., blind testing of materials with known values, other external assessment programs, split samples with another laboratory instrument or method, comparison with Kodachrome slides from a reference source)?

D5221

§493.1236 Standard: Evaluation of proficiency testing performance.

(d) All proficiency testing evaluation and verification activities must be documented.

Interpretative Guidelines §493.1236(d)

Documentation must include review of all unsatisfactory scores and the corrective action taken.

D5291

§493.1239 Standard: General laboratory systems quality assessment.

(a) The laboratory must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess, and, when indicated, correct problems identified in the general laboratory systems requirements specified at §§493.1231 through 493.1236.

Interpretative Guidelines §493.1239(a)-(c)

Quality Assessment (QA) is an ongoing review process that encompasses all facets of the laboratory's technical and non-technical functions and all locations/sites where testing is performed. QA also extends to the laboratory's interactions with responsibilities to patients, physicians, and other laboratories ordering tests, and the other non-laboratory areas or departments of the facility of which it is a part.

When the laboratory discovers an error or identifies a potential problem, actions must be taken to correct the situation. This correction process involves identification and resolution of the problem, and development of policies that will prevent recurrence. Policies for preventing problems that have been identified must be written as well as communicated to the laboratory personnel and other staff, clients, etc., as appropriate. Over time, the laboratory must monitor the corrective action(s) to ensure the action(s) taken have prevented recurrence of the original problem.

All pertinent laboratory staff must be involved in the assessment process through discussions or active participation.

QA of the General Laboratory System includes assessing practices/issues related to:

- Patient confidentiality;
- Specimen identification and integrity;
- Complaint investigations;
- Communications;
- Personnel competency; and
- Proficiency testing performance.

An example would be monitoring the type and number of complaints received by the laboratory such as a particular client continuously complaining about the laboratory's failure to promptly respond to STAT test requests. The laboratory must have documentation that the complaint was investigated and appropriate action taken to correct the problem.

Verify that the laboratory has a system in place for monitoring and evaluating confidentiality of patient information.

Probes §493.1239(a)

How does the laboratory assure that an individual who had problems in performance is competent after appropriate retraining and technical assistance is completed?

How does the laboratory determine which complaints require investigation and which do not?

D5293

§493.1239 Standard: General laboratory systems quality assessment.

(b) The general laboratory systems quality assessment must include a review of the effectiveness of corrective actions taken to resolve problems, revision of policies and procedures necessary to prevent recurrence of problems, and discussion of general laboratory systems quality assessment reviews with appropriate staff.

Interpretative Guidelines §493.1239(b)

Review assessment policies, procedures and reports to verify that the laboratory has a system in place to ensure continuous improvement. Corrective action reports are one indication that the laboratory is monitoring and evaluating laboratory performance and the quality of services.

Probes §493.1239(b)

When problems are identified in personnel competency, what corrective actions are instituted to assist employees to improve performance?

When the laboratory identifies a problem, are corrective actions taken, the resolution documented and monitored for effectiveness?

How does the laboratory prevent reoccurrences of problems?

How does the laboratory document and identify potential communication problems and corrective actions taken (e.g., with staff, referral laboratories)?

Have the corrective actions taken as a result of failures in proficiency testing (PT) and/or verification of accuracy testing as required under subpart H, improved performance?

(c) The laboratory must document all general laboratory systems quality assessment activities.

Interpretative Guidelines §493.1239(c)

The steps taken by the laboratory to identify and correct problems, and prevent their recurrences must be documented. All laboratory policies amended due to its QA activities must be noted.

D5300

Preanalytic Systems

§493.1240 Condition: Preanalytic systems.

Each laboratory that performs nonwaived testing must meet the applicable preanalytic system(s) requirements in §§493.1241 and 493.1242, unless HHS approves a procedure, specified in Appendix C of the State Operations Manual #7, that provides equivalent quality testing. The laboratory must monitor and evaluate the overall quality of the preanalytic systems and correct identified problems as specified in §493.1249 for each specialty and subspecialty of testing performed.

Interpretive Guidelines §493.1240

Preanalytic refers to all steps taken prior to the actual testing of a patient specimen from the test request to the actual testing of the specimen. The preanalytic systems requirements

include three distinct sections: test requests; specimen submission, handling, and referral; and preanalytic systems quality assessment.

Significant deficiencies cited under this condition may indicate deficiencies under personnel responsibilities. Use D5300 when deficiencies are identified that have the potential to, or adversely affect patient testing, are systemic and pervasive throughout the laboratory, and are not limited to any one specialty or subspecialty.

To determine which tests are categorized as waived or nonwaived testing (i.e., moderate and high complexity tests), refer to the "Specific List For Categorization of Laboratory Test Systems, Assays, and Examinations by Complexity" [www.fda.gov/cdrh/cfia/index.html]. Test systems, assays and examinations not included in this listing (i.e., not yet categorized) are considered high complexity.

D5301

§493.1241 Standard: Test request.

(a) The laboratory must have a written or electronic request for patient testing from an authorized person.

Interpretative Guidelines §493.1241(a)

An "authorized person" means an individual authorized under State law to order tests or receive test results, or both. If the State law does not address who may order tests and receive test results, then anyone may order tests and receive test results. However, the laboratory must follow the requirements in the Social Security Act regarding test ordering for reimbursement under Medicare.

To assure only the authorized person is ordering the test, a laboratory using electronic test requests may issue passwords.

Use of standing orders should be clearly defined in written policy, describing which tests may be covered by standing orders and at what interval standing orders should be reconfirmed.

D5303

§493.1241 Standard: Test request.

(b) The laboratory may accept oral requests for laboratory tests if it solicits a written or electronic authorization within 30 days of the oral request and maintains the authorization or documentation of its efforts to obtain the authorization.

Interpretative Guidelines §493.1241(b)

Review the laboratory's policy for requesting written orders within 30 days of the oral requests. If no written order was received, verify the laboratory has documentation showing the attempt.

D5305

§493.1241 Standard: Test request.

(c) The laboratory must ensure the test requisition solicits the following information:

(c)(1) The name and address or other suitable identifiers of the authorized person requesting the test and, if appropriate, the individual responsible for using the test results, or the name and address of the laboratory submitting the specimen, including, as applicable, a contact person to enable the reporting of imminently life threatening laboratory results or panic or alert values.

Interpretive Guidelines §493.1241(c)(1)-(c)(8)

The address of the individual or laboratory requesting a test and/or using the test results should include the street, city or town and State of the individual or laboratory ordering or responsible for utilizing the test result. When appropriate, a telephone number or other mechanism to contact the individual responsible for using the test results should be provided to the laboratory.

If any information is missing from the test requisition or patient medical record or chart, the laboratory must determine whether to test the specimen. Laboratories must either obtain the missing information or report results and indicate on the test report, medical record or chart any limitations of test results due to the omission of patient information. If the missing information is essential (such as the family history for certain genetic tests) for accurate test results, it must be obtained prior to reporting patient test results.

Verify that test requisitions solicit all information necessary for the proper interpretation of results. This may include patient's age, sex, date and time of collection, specimen type (e.g., plasma, urine, spinal fluid), diagnosis, and date of last menstrual period (LMP) for Papanicolaou (PAP) smears. Verify that the instructions to clients are clear and specify the items that must be completed.

(c)(2) The patient's name or unique patient identifier.

(c)(3) The sex and age or date of birth of the patient.

(c)(4) The test(s) to be performed.

(c)(5) The source of the specimen, when appropriate.

(c)(6) The date and, if appropriate, time of specimen collection.

(c)(7) For Pap smears, the patient's last menstrual period, and indication of whether the patient had a previous abnormal report, treatment, or biopsy.

(c)(8) Any additional information relevant and necessary for a specific test to ensure accurate and timely testing and reporting of results, including interpretation, if applicable.

Interpretive Guidelines §493.1241(c)(8)

This may include such items as preventative or therapeutic medications, or family history.

Probes §493.1241(c)(1)-(c)(8)

How does the laboratory uniquely identify patient specimens that share the same or similar name, birth date, address or sex?

How does the requisition provide for inclusion of additional information when necessary (e.g., specimen type or source)?

D5307

§493.1241 Standard: Test request.

(d) The patient's chart or medical record may be used as the test requisition or authorization but must be available to the laboratory at the time of testing and available to CMS or a CMS agent upon request.

Probes §493.1241(d)

When the patient's chart or medical record is used as the test requisition does it provide all the information necessary to ensure accurate testing and reporting of results?

D5309

§493.1241 Standard: Test request.

(e) If the laboratory transcribes or enters test requisition or authorization information into a record system or a laboratory information system, the laboratory must ensure the information is transcribed or entered accurately.

Interpretive Guidelines §493.1241(e)

The laboratory must have an ongoing mechanism to ensure the accuracy of manual entries by personnel into an LIS.

How does the laboratory ensure that all individuals who enter data including clerical staff correctly match patient information?

D5311

§493.1242 Standard: Specimen submission, handling, and referral.

(a) The laboratory must establish and follow written policies and procedures for each of the following, if applicable:

(a)(1) Patient preparation.

Probes §493.1242(a)(1)

How does the laboratory ensure that all staff including phlebotomists gives appropriate instructions for patient preparation when needed?

Does the laboratory provide instructions directly to patients or to the client when proper patient preparation is required for optimal specimen collection? For example:

- *Proper preservation (temperature) and transportation time of semen specimens;*
- *Fasting instructions for lipid profile testing;*
- *Dietary restrictions prior to occult blood testing;*
- *Twenty-four hour urine collection for specific tests; and*
- *Fasting and two hour post-prandial glucose collections.*

If a patient has special communication (hearing impaired, not fluent in English, etc.) needs, are resources available to the client or to the patient, as appropriate, to ensure instructions for specimen collection, preservation, and transportation to the laboratory are properly understood?

Has the laboratory provided to its staff and/or individuals external to the laboratory who collect specimens, written procedures to ensure that patient preparation requirements have been followed?

(a)(2) Specimen collection.

Interpretive Guidelines §493.1242(a)(2)

Verify that procedures are available to the appropriate staff responsible for collecting the correct specimen, that personnel are using the appropriate collection technique (order and site of draw) and proper containers (e.g., acceptable anti-coagulant, sterile containers for culture specimens, dacron swabs vs. cotton swabs).

(a)(3) Specimen labeling, including patient name or unique patient identifier and, when appropriate, specimen source.

Interpretive Guidelines §493.1242(a)(3)

If the laboratory receives two specimens simultaneously with the same first and last name or birth date, the laboratory must have a system in place to process these specimens using distinct identifying indicators in order to distinguish between the specimens. This also pertains to personnel collecting and labeling specimens. This may include a system that involves labeling the specimen container and request slip (or the patient's medical record or chart) with a unique patient identification number, but does not preclude the use of other mechanisms to assist in patient identification and tracking of specimens throughout the collection, accessioning, testing, and reporting processes.

(a)(4) Specimen storage and preservation.

Interpretive Guidelines §493.1242(a)(4)

Review manufacturer's instructions for performance of each test method to ensure that specimens are properly stored (e.g., maintained at room temperature, kept refrigerated after separation, separated and frozen).

Probes §493.1242(a)(4)

What instructions are provided for specimen preservation and transportation, when applicable? For example:

- *Sputum for Cytology;*
- *Specimens for parathyroid hormone;*
- *Specimens for blood gas analysis;*
- *Specimens for urine culture and colony count; and*
- *Specimens for 24 hour urine collections requiring preservatives.*

(a)(5) Conditions for specimen transportation.

Probes §493.1242(a)(5)

Does the laboratory follow the manufacturer's or the referral laboratory's instructions, as appropriate, for transport of specimens?

(a)(6) Specimen processing.

Interpretive Guidelines §493.1242(a)(6)

Specimen processing may include receiving the specimen, accessioning the specimen, preparing the specimen for in-house analysis, preparation to send to a reference laboratory, preparing slides, and inoculating primary culture media, etc. Specimen processing also includes for: Parasitology: the fixation and concentration of specimens; Virology: the pretreatment of specimens with antibiotics, the manipulation of cell culture tubes and inoculation of the cell cultures prior to incubation; Mycobacteriology: performing digestion-decontamination and concentration procedures on clinical specimens; and Histopathology: fixation, embedding, cutting, mounting, staining and coverslipping.

Probes §493.1242(a)(6)

What policies or systems does the laboratory have in place to differentiate specimens that have similar names or identification information?

How does the laboratory recognize and process timed patient specimens (e.g., peaks, and troughs)?

(a)(7) Specimen acceptability and rejection.

Interpretive Guidelines §493.1242(a)(7)

Criteria for specimen acceptability and rejection must include the disposition of the rejected specimen(s). Use D5805. The laboratory should promptly notify the authorized person when a specimen meets its rejection criteria and is unsuitable for testing.

(a)(8) Specimen referral.

Interpretive Guidelines §493.1242(a)(8)

Ensure that the laboratory has a current service manual available for each reference laboratory that it uses that contains the reference laboratory's specimen requirements for the test to be performed.

Probes §493.1242(a)(8)

Are laboratory personnel familiar with procedures to prepare and/or submit specimens to the appropriate reference laboratory?

How does the laboratory ensure the security and preservation of specimens submitted to their reference laboratory (e.g., if the office closes before the arrival of the reference laboratory's courier)? How does the laboratory ensure a timely pick-up of specimens to be performed at the referral laboratory?

D5313

§493.1242 Standard: Specimen submission, handling, and referral.

(b) The laboratory must document the date and time it receives a specimen.

Interpretive Guidelines §493.1242(b)

When a sample is collected and a test is performed during the course of a patient's visit, the date and time recorded in the patient "sign-in" log may be used as the date and time of receipt into the laboratory.

D5315

§493.1242 Standard: Specimen submission, handling, and referral.

(c) The laboratory must refer a specimen for testing only to a CLIA-certified laboratory or a laboratory meeting equivalent requirements as determined by CMS.

Interpretive Guidelines §493.1242(c)

Some examples of laboratories meeting equivalent requirements are those of the Veterans Administration (VA), the Department of Defense (DOD) facilities, and CLIA-exempt laboratories.

Probes §493.1242(c)

How does the laboratory ensure that the reference laboratory has and maintains a current CLIA certificate?

D5317

§493.1242 Standard: Specimen submission, handling, and referral.

(d) If the laboratory accepts a referral specimen, written instructions must be available to the laboratory's clients and must include, as appropriate, the information specified in paragraphs (a)(1) through (a)(7) of this section.

Interpretive Guidelines §493.1242(d)

Ensure the laboratory has provided written instructions to each client that sends specimens/test requests. The instructions may contain information on specimen handling (e.g., collection, preservation, storage, transport, testing schedule times and how to obtain additional assistance for unusual circumstances).

D5391

§493.1249 Standard: Preanalytic systems quality assessment.

(a) The laboratory must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess, and when indicated, correct problems identified in the preanalytic systems specified at §§493.1241 through 493.1242.

Interpretive Guidelines §493.1249(a)-(c)

Quality Assessment (QA) is an ongoing review process that encompasses all facets of the laboratory's technical and non-technical functions and all locations/sites where testing is performed. QA also extends to the laboratory's interactions with and responsibilities to patients, physicians, and other laboratories ordering tests, and the other non-laboratory areas or departments of the facility of which it is a part.

When the laboratory discovers an error or identifies a potential problem, actions must be taken to correct the situation. This correction process involves identification and resolution of the problem, and development of policies that will prevent recurrence. Policies for preventing problems that have been identified must be written as well as communicated to the laboratory personnel and other staff, clients, etc., as appropriate. Over time, the laboratory must monitor the corrective action(s) to ensure the action(s) taken have prevented recurrence of the original problem. All pertinent laboratory staff must be involved in the assessment process through discussions or active participation.

*QA of the **Preanalytic System** includes assessing practices/issues related to test requests, specimen submission, handling and referral.*

Some examples include: monitoring the frequency of specimen handling problems (such as the use of an improper blood collection tube, inadequate mixing of blood specimens with anticoagulant after collection), and delays in specimen transport; identifying clients who repeatedly refer unacceptable specimens or improperly complete requisition forms and documentation of its efforts to reduce the recurrence of these problems.

Review assessment policies, procedures and reports to verify that the laboratory has a system in place to ensure continuous improvement. Corrective action reports are one indication that the laboratory is monitoring and evaluating laboratory performance and the quality of services.

Probes §493.1249(a)-(c)

When a laboratory uses off-site drawing facilities, what policies or procedures does the laboratory use to ensure proper accountability or tracking of patient specimens from time of collection to receipt by the laboratory performing the tests?

Does the laboratory perform periodic or spot checks for accurate transfer of information

(e.g., manual entries by personnel from test orders to test requisition or into an LIS)? For referral specimens, how does the laboratory check for transcription errors when patient test information is transcribed from the laboratory's original requisition form to the reference laboratory's requisition?

What actions does the laboratory take if test requisitions from one or more clients are consistently incomplete, illegible or contain incorrect information?

What actions does the laboratory take if specimens received from one client are consistently unsatisfactory for testing (e.g., specimens for Cytology)? Has the laboratory's efforts to reduce the recurrence of these problems been documented and effective?

D5393

§493.1249 Standard: Preanalytic systems quality assessment.

(b) The preanalytic systems assessment must include a review of the effectiveness of corrective actions taken to resolve problems, revision of policies and procedures necessary to prevent recurrence of problems, and discussion of preanalytic systems quality assessment reviews with appropriate staff.

(c) The laboratory must document all preanalytic systems quality assessment activities.

Interpretative Guidelines §493.1249(c)

The steps taken by the laboratory to identify and correct problems and prevent their recurrence must be documented. All laboratory policies amended due to its QA activities must also be noted.

D5400

Analytic Systems

§493.1250 Condition: Analytic Systems.

Each laboratory that performs nonwaived testing must meet the applicable analytic systems requirements in §§493.1251 through 493.1283, unless HHS approves a procedure, specified in Appendix C of the State Operations Manual #7, that provides equivalent quality testing. The laboratory must monitor and evaluate the overall quality of the analytic systems and correct identified problems as specified in §493.1289 for each specialty and subspecialty of testing performed.

NOTE: Throughout the analytic systems section, the regulations require laboratories to follow test system manufacturer's instruction for performing the testing. This means the laboratory must perform and follow the manufacturer's package insert as approved or cleared by the FDA.

Interpretive Guidelines §493.1250

Significant deficiencies cited under this condition may indicate deficiencies under personnel. Use D5400 when deficiencies are identified that are significant and have the potential to, or adversely affect patient testing, are systemic and pervasive throughout the laboratory, and are not limited to any one specialty or subspecialty.

Refer to §§493.1261 - 493.1278 for additional requirements for Bacteriology, Mycobacteriology, Mycology, Parasitology, Virology, Routine Chemistry, Hematology,

D5401

§493.1251 Standard: Procedure Manual.

(a) A written procedures manual for all tests, assays, and examinations performed by the laboratory must be available to, and followed by, laboratory personnel. Textbooks may supplement but not replace the laboratory's written procedures for testing or examining specimens.

Interpretive Guidelines §493.1251(a)

Procedures may be organized in the form of manuals, stored in computers and/or card files. Use D5403, if the procedure manual lacks any of the applicable information as specified in §493.1251(b)(1)-(14). If the laboratory has procedures that are not used for test performance, but are used for reference purposes, they may be placed in a reference section. You need not review reference procedures unless problems are identified with patient test results.

Centers for Disease Control and Prevention (CDC) and Armed Forces Institute of Pathology (AFIP) manuals, manufacturer's operating instructions, and package inserts, are acceptable provided the policies and procedures are available, and the methods in use are clearly indicated. If the laboratory modifies any procedure, the modification must be documented and verified/established as specified in §493.1253.

Probes §493.1251(a)

How does the laboratory ensure that personnel follow the procedures in the procedure manual? How are changes in procedures communicated to laboratory personnel? For competency issues, use D6030 or D6103 as applicable.

D5403

§493.1251 Standard: Procedure Manual.

(b) The procedure manual must include the following when applicable to the test procedure:

(b)(1) Requirements for patient preparation; specimen collection, labeling, storage, preservation, transportation, processing, and referral; and criteria for specimen acceptability and rejection as described in §493.1242.

Interpretive Guidelines §493.1251(b)(1)

If testing is delayed or not performed daily, specimens must be preserved or stored in accordance with the laboratory's procedures to assure specimen integrity.

Determine if the laboratory has a procedure for handling and identifying aliquotted specimens; e.g., sputum sent for Mycobacteriology and Cytology examinations; stool specimens for occult blood, routine culture, parasitology and C.difficile toxin assay; and cerebrospinal fluids for cell count, culture, glucose and protein.

(b)(2) Microscopic examination, including the detection of inadequately prepared slides.

(b)(3) Step-by-step performance of the procedure, including test calculations and interpretation of results.

(b)(4) Preparation of slides, solutions, calibrators, controls, reagents, stains, and other materials used in testing.

(b)(5) Calibration and calibration verification procedures.

Interpretive Guidelines §493.1251(b)(5)

Calibration and calibration verification procedures must be established in accordance with §493.1255.

(b)(6) The reportable range for test results for the test system as established or verified in §493.1253.

(b)(7) Control procedures.

Interpretive Guidelines §493.1251(b)(7)

Determine if the laboratory's quality control procedures include the following:

- *Type of control (e.g., manufacturer or in-house, electronic);*
- *Identity (e.g., normal, abnormal, level I, II, patient or a control);*
- *Number and frequency of testing controls;*
- *Control limits established in accordance with §§493.1253 and 493.1256; and*
- *Criteria to determine acceptable control results.*

(b)(8) Corrective action to take when calibration or control results fail to meet the laboratory's criteria for acceptability.

Interpretive Guidelines §493.1251(b)(8)

Ensure that corrective action procedures are established in accordance with §493.1282(b)(2).

(b)(9) Limitations in the test methodology, including interfering substances.

(b)(10) Reference intervals (normal values).

(b)(11) Imminently life-threatening test results, or panic or alert values.

(b)(12) Pertinent literature references.

(b)(13) The laboratory's system for entering results in the patient record and reporting patient results including, when appropriate, the protocol for reporting imminent life threatening results, or panic, or alert values.

Interpretive Guidelines §493.1251(b)(13)

Ensure the procedure manual provides instructions for reporting the patient's test results in the appropriate units or terminology. Use D5805.

Probes §493.1251(b)(13)

Do laboratory procedures address the process for reporting (oral and written) results on patients with multiple laboratory encounters to ensure that the exact name, date, time and identification of specimen is conveyed to the authorized person?

(b)(14) Description of the course of action to take if a test system becomes inoperable.

Interpretive Guidelines §493.1251(b)(14)

Laboratory information systems (LIS) procedures must be available to operators. Instructions should identify the individual(s), either by name or position, to notify if the LIS goes down or if a system error occurs.

Probes §493.1251(b)(14)

When the primary testing system is inoperable, what procedure does the laboratory use to bring the backup system on line?

D5405

§493.1251 Standard: Procedure Manual.

c) Manufacturer's test system instructions or operator manuals may be used, when applicable, to meet the requirements of paragraphs (b)(1) through (b)(12) of this section. Any of the items under paragraphs (b)(1) through (b)(12) of this section not provided by the manufacturer must be provided by the laboratory.

D5407

§493.1251 Standard: Procedure Manual.

(d) Procedures and changes in procedures must be approved, signed, and dated by the current laboratory director before use.

Interpretive Guidelines §493.1251(d)

Verify that the methods in the procedure manual are current for tests offered by the laboratory (e.g., reagent test kits and instruments used in the laboratory correlate with methods in the procedure manual).

All laboratory procedures including CDC and AFIP manuals, manufacturer's operator manuals, and package inserts must reflect the director's review and approval including any modifications in the procedure.

Approval of procedures is the responsibility of the laboratory director. A coversheet may be used for the director to approve the manual. Annual review of procedures is not required.

D5409

§493.1251 Standard: Procedure Manual.

e) The laboratory must maintain a copy of each procedure with the dates of initial use and discontinuance as described in §493.1105(a)(2).

D5411

§493.1252 Standard: Test systems, equipment, instruments, reagents, materials, and supplies.

a) Test systems must be selected by the laboratory. The testing must be performed following the manufacturer's instructions and in a manner that provides test results within the laboratory's stated performance specifications for each test system as determined under §493.1253.

Interpretive Guidelines §493.1252(a)

Following manufacturer's instructions means that the laboratory complies with the recommendations, suggestions and requirements in package inserts and/or instrument operator manuals. These include, but are not limited to:

- Handling reagents, materials, and supplies;*
- Adhering to conditions for storage and testing; and*
- Performing equipment maintenance and function checks.*

For Immunology tests such as Syphilis Serology, check for the following parameters:

- *Antigen volume;*
- *Incubation time and temperature;*
- *Light source;*
- *Rotator speed and circumference; and*
- *Conjugate titer.*

Probes §493.1252(a):

Are instruments with adjustable settings appropriately set for each substance or cell to be analyzed (e.g., gamma counters, flow cytometry)?

D5413

§493.1252 Standard: Test systems, equipment, instruments, reagents, materials, and supplies.

(b) The laboratory must define criteria for those conditions that are essential for proper storage of reagents and specimens, accurate and reliable test system operation, and test result reporting. The criteria must be consistent with the manufacturer's instructions, if provided. These conditions must be monitored and documented and, if applicable, include the following:

(b)(1) Water quality.

(b)(2) Temperature.

(b)(3) Humidity.

(b)(4) Protection of equipment and instruments from fluctuations and interruptions in electrical current that adversely affect patient test results and test reports.

Interpretive Guidelines §493.1252(b)

Water quality is classified by several different organizations, such as NCCLS, into different reagent grades dependent on microbial content, resistivity, silicate content, and particulate matter. Each laboratory is expected to use the appropriate water quality as required for each instrument, kit, or test system. Laboratories producing water should consider parameters such as pH, silicate content, particulate matter, and bacterial and organic content in assessing water quality. These parameters vary by test system and should be assessed by the laboratory for appropriateness and monitoring. Laboratories purchasing water that has already been classified are not expected to evaluate the above parameters unless specified by the manufacturer or by the laboratory in its procedure manual.

Temperature-controlled spaces, equipment, and instruments must be monitored and results documented for acceptable temperature ranges. Corrective action is needed when acceptable temperature ranges are exceeded. Use D5757.

Continuous monitoring of temperatures by a recording thermograph is acceptable provided the data and time of use are annotated. The charts must be retained to document that temperatures were within the limits established by the laboratory.

In lieu of manual temperature recording, it is acceptable for temperatures to be maintained and monitored internally by the instrument, provided either test results are flagged or not generated when the temperature range for test performance is exceeded.

Probes §493.1252(b)(1)-(b)(4)

How does the laboratory provide special conditions when required for specimen or reagent storage?

How is room temperature and humidity monitored when necessary for test performance, proper operation of reagents, instruments, equipment, or laboratory computer systems?

When temperatures and/or humidity are outside acceptable limits, how does the laboratory rectify the problem?

How does the laboratory that moves from testing site to testing site demonstrate that the conditions necessary for quality testing are maintained?

When mobile laboratory or temporary testing site equipment is not in use (weekends, overnight) how are instruments, reagents, stains, and other solutions protected from extreme temperature fluctuations?

D5415

§493.1252 Standard: Test systems, equipment, instruments, reagents, materials, and supplies.

(c) Reagents, solutions, culture media, control materials, calibration materials, and other supplies, as appropriate, must be labeled to indicate the following:

(c)(1) Identity and when significant, titer, strength or concentration.

(c)(2) Storage requirements.

(c)(3) Preparation and expiration dates.

Interpretive Guidelines §493.1252(c)(3)

Expiration dates for test kits and/or reagents may differ due to date opened or storage conditions (e.g., refrigerator, room temperature). Verify that laboratory personnel are aware of these differences and document the appropriate expiration date.

(c)(4) Other pertinent information required for proper use.

D5417

§493.1252 Standard: Test systems, equipment, instruments, reagents, materials, and supplies.

(d) Reagents, solutions, culture media, control materials, calibration materials, and other supplies must not be used when they have exceeded their expiration date, have deteriorated, or are of substandard quality.

Interpretive Guidelines §493.1252(d)

In citing deficiencies, for outdated or deteriorated materials, indicate whether these materials have been used for patient testing. Also, look for contamination, drying or other signs of deterioration. This is as important as checking expiration dates.

The laboratory must comply with the Food and Drug Administration (FDA) product dating requirements of 21 CFR 610.53 for blood products and other biologicals, and labeling requirements, as cited in 21 CFR 809.10 for all other in vitro diagnostics. Any exception to the product dating requirements in 21 CFR 610.53 will be granted by the FDA in the form of an amendment of the product license, in accordance with 21 CFR 610.53(d). All exceptions must be documented by the laboratory.

D5419

§493.1252 Standard: Test systems, equipment, instruments, reagents, materials, and supplies.

(e) Components of reagent kits of different lot numbers must not be interchanged unless otherwise specified by the manufacturer.

Interpretive Guidelines §493.1252(e)

"Kit" means all components of a test that are packaged together.

§493.1253 Standard: Establishment and verification of performance specifications.

(a) Applicability. Laboratories are not required to verify or establish performance specifications for any test system used by the laboratory before April 24, 2003.

Interpretive Guidelines §493.1253(a)

The requirements of §493.1253 apply to each nonwaived test system (i.e., moderate and high complexity) introduced into the laboratory on or after April 24, 2003. This includes the following:

- A test system that is introduced into the laboratory for the first time to measure an analyte that the laboratory has not previously measured;*
- A test system introduced for the first time into the laboratory for a test that the laboratory currently performs on an alternative test system (e.g., instrument A has been used to perform cholesterol testing, now instrument B will be used);*
- An analyte added to a test system that can measure multiple analytes which the laboratory has been using for patient testing but has not previously reported patient results for this particular analyte; and*
- A modification to a test system that the laboratory has been using for patient testing (e.g., the laboratory reduces the specimen and/or reagent volumes).*

When multiple instruments (including the same make and model, e.g., point-of-care instruments) are used to perform the same test, the laboratory must verify or establish, as applicable, performance specifications for each instrument.

Refer to requirements in subpart M, for training and competency of personnel.

Prior to testing patient specimens, the laboratory should begin the process of establishing the assay or test system's performance specifications by performing the following:

- Obtain and review test performance data from the test development source (e.g., Centers for Disease Control and Prevention), including number of tests performed on different patient groups or specimen types, preliminary data on sensitivity, specificity, and accuracy, precision, interfering substances, etc.*
- Use testing personnel that have been trained and demonstrated competency performing the same methodology for other agents.*

As soon as control/calibration materials become available, the applicable requirements in §493.1253 must be met.

Specific information regarding testing for agents of emergent public health significance and alternative methods /procedures for establishing performance specifications may be found at www.cdc.gov.

NOTE: Public health testing performed on environmental (non-human) samples is not subject to CLIA.

D5421

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(1) Verification of performance specifications. Each laboratory that introduces an unmodified, FDA-cleared or approved test system must do the following before reporting patient test results:

Interpretive Guidelines §493.1253(b)(1)

The laboratory is responsible for verifying the performance specifications of each nonwaived unmodified FDA-cleared or approved test system that it introduces, prior to reporting patient test results. The verification of method performance should provide evidence that the accuracy, precision, and reportable range of the procedure are adequate to meet the clients' needs, as determined by the laboratory director and clinical consultant. A laboratory may use the manufacturer's performance specifications as a guideline, but is responsible for verifying the manufacturer's analytical claims before initiating patient testing.

*When a temporary replacement (loaner) instrument is received which is identical (i.e., same make and model, and method for the same analyte) to the instrument which is being replaced, the laboratory must verify comparable performance by comparing, at a minimum, results of two or more levels of controls **and** either previously tested proficiency samples or previously tested patient specimens.*

If a method was verified by someone other than the laboratory staff (e.g., manufacturer representative), the laboratory must demonstrate that this verification correlates with its in-house test performance. This may be accomplished by the laboratory testing "known" samples.

For some qualitative tests, the laboratory may verify the manufacturer's specifications by testing known positive and negative samples to assure that the expected results are obtained. (Specimens of known quantitative value may be used to verify the accuracy of a qualitative test.)

Prior to introducing a test for routine patient testing, the laboratory must review and evaluate the verification data.

Each laboratory is responsible for determining that its performance specifications for each test system are not affected by the relocation of the laboratory or test system. (See manufacturer's package insert regarding critical requirements such as set-up, limitations, environmental conditions, etc.)

If calibration material is used to verify method performance specifications, the laboratory must demonstrate that there is a minimal matrix effect and the calibration material is appropriate for verifying test system performance specifications.

If the LIS performs any calculations to determine a laboratory result, the calculations must be verified immediately after the LIS is programmed and prior to initial calculation of patient results.

"Less than" is used for reporting test results that are below the laboratory's detection limits for an analyte. (Detection limits must be established through method verification.) "Equivalent designation" is used to report test results for those methods that yield results

below a clinically significant level (e.g., for a quantitative immunology test, patient results may be clinically negative at a 1:8 titer and test results may be reported as "1:8 negative). (The normal value is 1:8 or less.) "Greater than" is used for reporting test results that are above the laboratory's detection limits for an analyte. If patient test results exceed the laboratory's reportable range, the laboratory must report the result as greater than the highest detection limit, reassay a diluted patient specimen and report the calculated result, or send the specimen to a reference laboratory.

Probes §493.1253(b)(1)

How does the laboratory determine if a new or revised LIS program (whether purchased or developed in-house) performs acceptably before it is integrated into routine operation?

(b)(1)(i) Demonstrate that it can obtain performance specifications comparable to those established by the manufacturer for the following performance characteristics:

Interpretive Guidelines §493.1253(b)(1)(i)

Laboratories may simultaneously verify multiple performance specifications by choosing appropriate samples; e.g., repeatedly test (precision) samples with known (accuracy) high and low values (reportable range). This testing should be performed among all operators on different days. In addition, for test systems of the same make and model, consider verifying performance specifications of these devices at the same time.

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(1)(i)(A) Accuracy.

Interpretive Guidelines §493.1253(b)(1)(i)(A)

Accuracy- *The laboratory is responsible for verifying that the method produces correct results. Verification of accuracy, may be accomplished by:*

- *Testing reference materials;*
- *Comparing results of tests performed by the laboratory against the results of a reference method; or*
- *Comparing split sample results with results obtained from a method, which is shown to provide clinically valid results.*

For qualitative methods, the laboratory must verify that a method will identify the presence/absence of the analyte.

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(1)(i)(B) Precision.

Interpretive Guidelines §493.1253(b)(1)(i)(B)

Precision (Reproducibility)- *The laboratory is responsible for verifying the precision of each test system by assessing day-to-day, run-to-run, and within-run variation, as well as operator variance. This may be accomplished by:*

- *Repeat testing of known patient samples over time;*
- *Testing QC material in duplicate and over time; or*
- *Repeat testing of calibration materials over time.*

EXCEPTION: For fully automated systems that are not user dependent, operator variance does not need to be evaluated.

(b)(1)(i)(C) Reportable range of test results for the test system.

Interpretive Guidelines §493.1253(b)(1)(i)(C)

Reportable Range- The laboratory is responsible for verifying the reportable range of patient test results for each test system. Verification of reportable range, may be accomplished by:

- Assaying low and high calibration materials or control materials; or
- Evaluating known samples of abnormal high and abnormal low values.

Hematology whole blood high range calibration materials are not generally available. Therefore, laboratories may use patient specimens with verified elevated cell counts to verify the upper limit of the reportable range.

Probes §493.1253(b)(1)(i)(c)

If a dilution procedure is used when patient results exceed the test system's reportable range, how does the laboratory assure the appropriate diluent is used for each type of specimen?

How does the laboratory verify and document the accuracy of the results for diluted specimens?

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(1)(ii) Verify that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population.

Interpretive Guidelines §493.1253(b)(1)(ii)

Reference Range (Normal Values) - The laboratory may use the manufacturer's reference range provided it is appropriate for the laboratory's patient population (i.e., a normal range that reflects the type of specimen and demographic variables such as age and sex, as applicable). If the manufacturer has not provided reference ranges appropriate for the laboratory's patient population, the laboratory may use published reference range(s). The laboratory must evaluate an appropriate number of specimens to verify the manufacturer's claims for normal values or, as applicable, the published reference ranges.

D5423

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(2) Establishment of performance specifications. Each laboratory that modifies an FDA-cleared or approved test system, or introduces a test system not subject to FDA clearance or approval (including methods developed in-house and standardized methods such as text book procedures, or uses a test system in which performance specifications are not provided by the manufacturer must, before reporting patient test results, establish for each test system the performance specifications for the following performance characteristics, as applicable:

Interpretive Guidelines §493.1253(b)(2)

Prior to reporting patient test results, the laboratory is responsible for establishing the performance specifications for each modified FDA-cleared or approved test system, each test system not subject to FDA clearance or approval, and each test system for which the manufacturer does not provide performance specifications. The establishment of method performance specifications should provide evidence that the accuracy, precision, analytical sensitivity, and analytical specificity of the procedure is adequate to meet the clients' needs as determined by the laboratory director and clinical consultant.

"Modified by the laboratory," means any change to the assay that could affect its performance specifications for sensitivity, specificity, accuracy, or precision, etc. Laboratory modification of the manufacturer's instructions that could affect performance specification's include but are not limited to:

- *Change in specimen handling instructions;*
- *Incubation times or temperatures;*
- *Change in specimen or reagent dilution;*
- *Using a different calibration material (or changing the manufacturer's set-points);*
- *Introducing a different antibody (source, monoclonal-vs-polyclonal);*
- *Change or elimination of a procedural step;*
- *Change or addition of detector (conjugate) or substrate;*
- *Change in the solid phase;*
- *Change in the cutoff or method of calculating the cutoff for semi-quantitative assays;*
- *Change in the endpoint or calculation of the endpoint;*
- *Addition of adsorbent;*
- *Change in the strain of antigen in serologic assays; and*
- *Changing the calibrator/reference material.*

EXCEPTIONS: Use of reagents that are exempt from the premarket notification procedures in 21 CFR 807 for an instrument produced by another manufacturer is not considered a method modification. If the FDA has cleared a manufacturer's reagents and/or calibration materials for use with an instrument produced by another manufacturer, the use of these reagents/materials is not considered a method modification and does not require establishment of performance specifications. However, the laboratory must verify performance specifications as required under §493.1253(b)(1). Verification of performance specifications is required if reagents are changed to those of another manufacturer.

"Modified by the laboratory," also means any change in intended use that could affect test system performance specifications for sensitivity, specificity, accuracy, and precision, etc., and the clinical utility of the test system. Changes in intended use are considered "off-label" use of a commercial test system. CAUTION: "Off-label" use is not supported by the manufacturer's clinical data and when identified, must be reported to the FDA.

Examples of changes in intended use are:

- *Using a different sample matrix (plasma vs. urine);*
- *Using or promoting the test for another purpose (screening vs. diagnostic); and*
- *Changing the type of analysis (qualitative results reported as quantitative).*

NOTE: *Because analyte specific reagents (ASR) are not approved by the FDA, the laboratory is responsible for establishing performance specifications for the test systems using these reagents.*

For automated or semi-automated analyzers, reprocessed (reconditioned) rotors/cuvettes which have passed quality control inspection criteria of the reprocessing company, and returned to the same laboratory that sent them for cleaning and re-use, is not considered a method modification.

Specimens of known quantitative value may be used to determine the laboratory's performance specifications for a qualitative test.

Each laboratory is responsible for determining that its performance specifications for each test method are not affected by the relocation of the laboratory or test system. (See

manufacturer's package insert regarding critical requirements such as set-up, limitations, environmental conditions, etc.)

If calibration material is used to establish method performance specifications, the laboratory must demonstrate that there is a minimal matrix effect and the calibration material is appropriate for establishing test system performance specifications.

If the LIS performs any calculations to determine a laboratory result, the calculations must be verified immediately after the LIS is programmed and prior to initial calculation of patient results.

CMS policies for the establishment of performance specifications for new test assays or test systems used to detect agents of emergent public health significance will be forthcoming in future publications.

NOTE: *Public health testing performed on environmental (non-human) samples is not subject to CLIA.*

Probes §493.1253(b)(2)

How does the laboratory determine if a new or revised LIS program (whether purchased or developed in-house) performs acceptably before it is integrated into routine operation?

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(2)(i) Accuracy.

Interpretive Guidelines §493.1253(b)(2)(i)

Accuracy-

The laboratory is responsible for establishing that the method produces correct results.

Establishment of accuracy, may be accomplished by:

- *Testing reference materials or comparing results of tests performed using an established reference method; or*
- *Comparing split sample results with results obtained from a method, which is shown to provide clinically valid results.*

For qualitative methods, the laboratory is responsible for establishing that a method will identify the presence/absence of the analyte.

In establishing a test system for a new analyte, research results may be used to document the accuracy of the test by correlation with the clinical presentation. In addition, the laboratory needs to determine the test system's precision and have mechanisms for determining analytical specificity, analytical sensitivity, and interfering substances.

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(2)(ii) Precision.

Interpretive Guidelines §493.1253 (b)(2)(ii)

Precision (Reproducibility)- *The laboratory is responsible for establishing the precision of each test system by assessing day-to-day, run-to-run, and within-run variation, as well as operator variance.*

This may be accomplished by:

- *Repeat testing of known patient samples over time;*

- Testing QC material in duplicate and over time; or
- Repeat testing of calibration materials over time.

EXCEPTION: For fully automated systems that are not user dependent, operator variance does not need to be evaluated.

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(2)(iii) Analytical sensitivity.

Interpretive Guidelines §493.1253(b)(2)(iii)

Analytical Sensitivity- The laboratory is responsible for determining the lowest concentration or amount of the analyte or substance that can be measured or distinguished from a blank, i.e., minimum detection limits or how much of the analyte must be present to be measured.

For modified test systems, the laboratory may use the lower limit of the manufacturer's reportable range if it has demonstrated that the modification has not affected the lower limit.

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(2)(iv) Analytical specificity to include interfering substances.

Interpretive Guidelines §493.1253(b)(2)(iv)

Analytical Specificity - The laboratory must determine the extent to which the method measures the analyte for which it is reporting results.

Interfering Substances- The laboratory must document information regarding interfering substances from product information, literature, or its own testing. These may include: specimen hemolysis, anticoagulant, lipemia, and turbidity; patients' clinical conditions, disease states, and medications.

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(2)(v) Reportable range of test results for the test system.

Interpretive Guidelines §493.1253(b)(2)(iv)

Reportable Range- The laboratory is responsible for establishing the upper and lower limits of the test system.

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(2)(vi) Reference intervals (normal values).

Interpretive Guidelines §493.1253(b)(2)(vi)

Reference Range (Normal Values) - The laboratory must establish a reference range that is appropriate for the laboratory's patient population (i.e., a normal range that reflects the type of specimen and demographic variables such as age and sex, as applicable).

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(2)(vii) Any other performance characteristic required for test performance.

§493.1253 Standard: Establishment and verification of performance specifications.

(b)(3) Determination of calibration and control procedures. The laboratory must determine the test system's calibration procedures and control procedures based upon the performance specifications verified or established under paragraph (b)(1) or (b)(2) of this section.

Interpretive Guidelines §493.1253(b)(3)

Through the verification/establishment process, the laboratory defines the frequency for calibration and control performance as well as the type, number, and concentration of calibration and control materials used to monitor, detect error, and evaluate method performance. The frequency for calibration and control performance must not be less than the frequency specified in the manufacturer's instructions.

In establishing the calibration and quality control frequency, the laboratory must consider:

- *Test system instrument/reagent stability, including relocation;*
- *Frequency with which the test is performed;*
- *Technique dependence of the method;*
- *Frequency of quality control failures; and*
- *Training, experience, and competency of technical personnel.*

For additional criteria in determining calibration and quality control frequency refer to §§493.1255 and 493.1256.

§493.1253 Standard: Establishment and verification of performance specifications.

(c) Documentation. The laboratory must document all activities specified in this section.

Interpretive Guidelines §493.1253(c)

The actual measurement(s) taken, reactions and/or observations must be recorded. Acceptable formats for documentation may vary.

§493.1254 Standard: Maintenance and function checks.

(a) Unmodified manufacturer's equipment, instruments, or test systems. The laboratory must perform and document the following:

Interpretative Guideline §493.1254(a)

When a laboratory introduces a new test system, the laboratory may determine, depending on the outcome of the performance specifications, that additional measures are necessary in order to ensure accurate and reliable test results.

D5429

§493.1254 Standard: Maintenance and function checks.

(a)(1) Maintenance as defined by the manufacturer and with at least the frequency specified by the manufacturer.

Interpretive Guidelines §493.1254(a)(1)

"As defined by the manufacturer " means that the laboratory complies with the maintenance recommended in package inserts and/or instrument operator manuals for

each piece of equipment/instrument it uses, including those that are peripherally involved in patient testing (e.g., incubators, centrifuges, safety cabinets, autoclaves and microscopes).

A laboratory's maintenance program is usually divided into two parts:

- *Unscheduled repairs when needed; and*
- *Scheduled preventive maintenance (PM), which is performed to prevent breakdowns or malfunctions, to prolong the life of an instrument and to maintain optimum operating characteristics.*

A service contract for PM from an outside source is acceptable provided that for each instrument or piece of equipment, there is a description of the service to be performed and frequency of service.

A service contract does not negate the laboratory's responsibility for performing other routine maintenance not included in the maintenance contract. Acceptable performance parameters (if applicable) must be documented.

The laboratory must perform and document maintenance as specified by the manufacturer for the LIS computer and devices such as monitors, printers and modems. All devices must be maintained to assure accurate, clear, and interference-free transmission.

Probes §493.1254(a)(1)

Are LIS system components (e.g., server, hard drives, disk packs) maintained according to the manufacturer's instructions?

When downtime is required to perform maintenance on LIS equipment, how are LIS users notified?

How does the laboratory's maintenance program assure that instruments and equipment maintain optimum operating characteristics and minimize breakdowns?

D5431

§493.1254 Standard: Maintenance and function checks.

(a)(2) Function checks as defined by the manufacturer and with at least the frequency specified by the manufacturer. Function checks must be within the manufacturer's established limits before patient testing is conducted.

Interpretive Guidelines §493.1254(a)(2)

Function checks refer to those activities performed to evaluate critical operating characteristics (e.g., stray light, zeroing, electrical levels, optical alignment, background counts, counting efficiency) according to the accepted method of operation for each type of device or instrument. Daily quality control activities and function checks are performed prior to patient testing to ensure that an instrument is functioning correctly and is properly calibrated (Checking electrical, mechanical, and operational functions may be independent of the procedure). The performance of daily quality control activities may serve as an additional instrument function check, since analysis of external control samples check the operating characteristics of a test system, including instrument stability and calibration.

The laboratory must follow and document the necessary functions checks as stated by the laboratory information system (LIS) manufacturer for the LIS computer and

devices such as monitors, printers and modems.

For instruments that automatically perform function checks and flag problems, the laboratory is required to document the corrective actions in response to the flagged problems. Use D5753 for deficiencies related to documenting corrective actions in response to the flagged problems.

Flow cytometry:

A fluorescence standard(s) for each fluorochrome should be used each day of patient testing to ensure:

- Proper alignment of the optical system;*
- Standardization of the fluorescence detectors;*
- Resolution of dimly-stained particles; and*
- Appropriate compensation for spectral overlap of the fluorochromes.*

Fluorescence standards should have the same fluorochromes as are used for the test, and with the exception of alignment standards, should have similar fluorescence intensities as found in the test specimens. The laboratory must have an acceptable range of performance for all procedures.

Probes §493.1254(a)(2)

For those methods in which the centrifugation is a critical portion of the test, does the laboratory check the RPM's and timing periodically (e.g., urine sediments)?

Do the records of the laboratory that moves from testing site to testing site demonstrate the performance of function checks as necessary?

In immunofluorescent test procedures, how does the laboratory assure that the bulb is emitting ultraviolet light at the correct wave length?

How does the laboratory ensure that the fluorescent light source has not exceeded the manufacturer's established optimal timeframe?

For procedures or test systems that require pipetting or dilution of patient specimens separately from controls or calibrators, how are autodiluters, microdiluters, and/or pipettors checked for adequate and consistent delivery?

For those systems that perform simultaneous fluid delivery to multi-well plates or tubes, how does the laboratory assure uniform delivery of reagents or washing solutions to all wells or tubes?

§493.1254 Standard: Maintenance and function checks.

(b) Equipment, instruments, or test systems developed in-house, commercially available and modified by the laboratory, or maintenance and function check protocols are not provided by the manufacturer. The laboratory must do the following:

Interpretive Guidelines §493.1254(b)

The laboratory must establish and follow procedures for performing maintenance and function checks on each piece of equipment/instrument it uses, including those that are peripherally involved in patient testing (e.g., incubators, centrifuges, safety cabinets, autoclaves and microscopes).

A manufacturer's instructions may not require maintenance and function checks, however, if the laboratory determines that a maintenance and/or function check protocol is necessary in order to ensure accurate and reliable test results, the laboratory must establish a maintenance protocol and perform and document the activities. Additionally, a laboratory must define the function check protocol and perform and document the function checks.

D5433

§493.1254 Standard: Maintenance and function checks.

(b)(1)(i) Establish a maintenance protocol that ensures equipment, instrument, and test system performance that is necessary for accurate and reliable test results and test result reporting.

(b)(1)(ii) Perform and document the maintenance activities specified in paragraph (b)(1)(i) of this section.

Interpretive Guidelines §493.1254(b)(1)

A laboratory's maintenance program is usually divided into two parts:

- *Unscheduled repairs when needed; and*
- *Scheduled preventive maintenance (PM) which is performed to prevent breakdowns or malfunctions, to prolong the life of an instrument and to maintain optimum operating characteristics.*

Probes §493.1254(b)(1)

How does the laboratory's maintenance program assure that instruments and equipment maintain optimum operating characteristics and minimize breakdowns?

Has the laboratory evaluated whether any modifications it has made to a manufacturer's instrument or piece of equipment has resulted in the need for additional maintenance or function checks, and, if so, have the additional procedures been established and implemented?

D5435

§493.1254 Standard: Maintenance and function checks.

(b)(2)(i) Define a function check protocol that ensures equipment, instrument, and test system performance that is necessary for accurate and reliable test results and test result reporting.

(b)(2)(ii) Perform and document the function checks, including background or baseline checks, specified in paragraph (b)(2)(i) of this section. Function checks must be within the laboratory's established limits before patient testing is conducted.

Interpretive Guidelines §493.1254(b)(2)(i)-(b)(2)(ii)

The laboratory must establish and follow procedures for performing function checks on each piece of equipment/instrument it uses, including those that are peripherally involved in patient testing (e.g., incubators, centrifuges, safety cabinets, autoclaves).

Function checks refer to those activities performed to evaluate critical operating characteristics (e.g., stray light, zeroing, electrical levels, optical alignment, background counts, counting efficiency) according to the accepted method of operation for each type of device or instrument. Daily quality control activities and function checks are performed prior to patient testing to ensure that an instrument is functioning correctly and is properly

calibrated. Checking electrical, mechanical, and operational functions may be independent of the procedure. The performance of daily quality control activities serves as an additional instrument function check, since analysis of external control samples check the operating characteristics of a test system, including instrument stability and calibration.

When function checks are critical to test performance, the laboratory must have a mechanism in place to monitor such items as:

- *Rotator speed and circumference;*
- *Timers;*
- *Anaerobic chambers;*
- *Cell washers;*
- *Radioactive particle counters;*
- *Blood cell counters; and*
- *Nucleic acid amplification equipment.*

Flow cytometry:

A fluorescence standard(s) for each fluorochrome must be used each day of patient testing to ensure:

- *Proper alignment of the optical system;*
- *Standardization of the fluorescence detectors;*
- *Resolution of dimly-stained particles; and*
- *Appropriate compensation for spectral overlap of the fluorochromes.*

Fluorescence standards must have the same fluorochromes incorporated into them as are used for the test, and with the exception of alignment standards, must have similar fluorescence intensities as found in the test specimens. The laboratory must have an acceptable range of performance for all procedures.

For flow cytometers with air-cooled lasers, the laser should be tested each day patients are tested by peaking the laser signal and monitoring the current input (amps) to laser light output (milliwatts) to determine whether the brewster windows are in need of cleaning.

Probes §493.1254(b)(2)

For those methods in which the centrifugation is a critical portion of the test, how has the laboratory checked the established RPM's and timing as necessary?

In immunofluorescent test procedures, how does the laboratory assure that the bulb is emitting ultraviolet light at the correct wave length?

If function checks are not required or recommended by the manufacturer, how does the laboratory establish the performance criteria of its equipment and instruments?

For RIA testing, are backgrounds or baselines measured for each setting? For example, if the laboratory uses more than one type of isotope, at what window setting are background counts performed and recorded?

When performing flow cytometry analysis using two or more fluorochromes simultaneously, how does the laboratory identify and adjust for "spill over" into the other fluorescence detectors?

Calibration and calibration verification procedures are required to substantiate the continued accuracy of the test system throughout the laboratory's reportable range of test results for the test system. Unless otherwise specified in this subpart, for each applicable test system the laboratory must do the following:

Interpretive Guidelines §493.1255

For definitions of calibration and calibration verification, refer to §493.2.

For calibration and calibration verification of blood gas analysis, see §493.1267(a) through (d).

In many instances, the performance of method calibration serves to satisfy the requirement for instrument calibration. Calibration procedures are not to be confused with instrument/equipment function checks, at §493.1254.

D5437

§493.1255 Standard: Calibration and calibration verification procedures

(a) Perform and document calibration procedures-

) Following the manufacturer's test system instructions, using calibration materials provided or specified, and with at least the frequency recommended by the manufacturer;

(a)(2) Using the criteria verified or established by the laboratory as specified in §493.1253(b)(3)--

(a)(2)(i) Using calibration materials appropriate for the test system and, if possible, traceable to a reference method or reference material of known value; and

(a)(2)(ii) Including the number, type, and concentration of calibration materials, as well as acceptable limits for and the frequency of calibration; and

(a)(3) Whenever calibration verification fails to meet the laboratory's acceptable limits for calibration verification.

Interpretive Guidelines §493.1255(a)

The calibration requirement does not apply to a variety of procedures, which include, but are not limited to:

- *Manual procedures not involving an instrument (e.g., microbiology cultures, Kirby-Bauer disk susceptibility tests, tilt-tube prothrombin time test systems, ABO group and D (Rho) typing);*
- *Microscopic procedures (e.g., KOH preparations, pinworm preparations, urine sediment analysis, all manual differential procedures, manual cytology screening procedures); and*
- *Procedures involving an instrument in which calibration is not practical, e.g., prothrombin procedures.*

Laboratories performing testing with instruments that cannot be adjusted (e.g., unit use devices), must follow the manufacturer's instructions for initial calibration and perform calibration verification as required at §493.1255(b).

The term "calibration material" has generally replaced "standard" since many instruments now use serum-based reference materials. "Calibration material" means a solution that has a known amount of analyte weighed in or has a value determined by repetitive testing using a reference or definitive test method. Calibration material may be traceable to a National Institute for Standards and Technology (NIST) Standard, if possible.

Test method calibration procedure is based on the manufacturer's recommendations and must be followed. However, if calibration proves less stable than the manufacturer's recommendation, additional calibration materials and/or more frequent calibration may be required, as established or verified by the laboratory under §493.1253(b)(3).

The actual measurement(s) taken, reactions and/or observations must be recorded.

Probes §493.1255(a)

If the laboratory calculates values for one or more calibration materials, are the calculations correct, and do the records reflect that the measured values are within the laboratory's established limits for the calibration materials?

D5439

§493.1255(b) Standard: Calibration and calibration verification procedures

(b) Perform and document calibration verification procedures—

(b)(1) Following the manufacturer's calibration verification instructions;

(b)(2) Using the criteria verified or established by the laboratory under §493.1253(b)(3)—

(b)(2)(i) Including the number, type, and concentration of the materials, as well as acceptable limits for calibration verification; and

(b)(2)(ii) Including at least a minimal (or zero) value, a mid-point value, and a maximum value near the upper limit of the range to verify the laboratory's reportable range of test results for the test system; and

(b)(3) At least once every 6 months and whenever any of the following occur:

(b)(3)(i) A complete change of reagents for a procedure is introduced, unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected by reagent lot number changes.

(b)(3)(ii) There is major preventive maintenance or replacement of critical parts that may influence test performance.

(b)(3)(iii) Control materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem.

(b)(3)(iv) The laboratory's established schedule for verifying the reportable range for patient test results requires more frequent calibration verification.

Interpretive Guidelines §493.1255(b)

If the laboratory performs a calibration protocol using 3 or more levels of calibration materials that include a low, mid, and high value at least every 6 months, the calibration verification requirement is met.

For kinetic enzymes, the calibration verification requirements may be met by verifying the procedure using a high enzyme level material such as a control, calibration material, or patient specimen and diluting it to cover the reportable range.

Control activities routinely used to satisfy the requirement for §493.1256 do not satisfy the calibration verification requirements.

EXCEPTIONS:

1. For automated cell counters, the calibration verification requirements are considered met if the laboratory follows the manufacturer's instructions for instrument operation and tests 2 levels of control materials each day of testing provided the control results meet the laboratory's criteria for acceptability.

2. If the laboratory follows the manufacturer's instruction for instrument operation and routinely tests three levels of control materials (lowest level available, mid-level, and highest level available) more than once each day of testing; the control material results meet the laboratory's criteria for acceptability and the control materials are traceable to National Institute of Standards and Technology (NIST) reference materials, the calibration verification requirements are met.

Calibration materials, proficiency testing samples with known results, or control materials with known values may be used to perform calibration verification. For these materials, the laboratory must define acceptable limits for the difference between the measured value obtained, versus the actual concentration of the materials.

If reagents are obtained from a manufacturer and all of the reagents for a test are packaged together, the laboratory is not required to perform calibration verification for each package of reagents, provided the packages of reagents are received in the same shipment and contain the same lot number.

When reviewing the laboratory's maintenance and function check records as required in §493.1254, determine whether the laboratory performed calibration verification when major maintenance occurred or critical parts were replaced.

The actual measurement(s) taken, reactions and/or observations must be recorded.

Probes §493.1255(b)

If a laboratory does not perform calibration verification after a complete change of reagents, what data does the laboratory have to document that changing reagent lot numbers does not affect the reportable range of patient test results, and does not adversely affect control results?

D5441

§493.1256 Standard: Control procedures.

- (a) For each test system, the laboratory is responsible for having control procedures that monitor the accuracy and precision of the complete analytic process.
- (b) The laboratory must establish the number, type, and frequency of testing control materials using, if applicable, the performance specifications verified or established by the laboratory as specified in §493.1253(b)(3).
- (c) The control procedures must-
 -) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance.
- (c)(2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance.

Interpretive Guidelines §493.1256(a)-(c)

For each test system, the laboratory is responsible for monitoring the accuracy and precision of each phase of the analytic testing process by using control procedures that will detect immediate errors and errors occurring over time. Errors may occur due to test system failure, change in environmental conditions, and operator performance.

TEST SYSTEM

Test system failures may result from reagent contamination or deterioration, reagent lot variation, reaction temperature fluctuations, inadequate sampling, improper or loss of calibration, electronic or mechanical failure, power supply

variances, etc.

ENVIRONMENT

Environmental conditions that may affect test system performance include temperature, airflow, light intensity, humidity, altitude, etc.

OPERATOR

Operator performance that may affect testing include improper specimen preparation and handling, incorrect test interpretation, failure to follow the manufacturer's test system instructions, etc. Operator training prior to testing is critical and competency assessment over time is necessary to ensure continued appropriate test performance. (See subpart M)

NCCLS EP-18, "Quality Management for Unit-Use Testing," provides guidance for identifying test system, environmental, and operator sources of error. Many manufacturers adhere to this NCCLS guidance and have identified potential sources of error for their test system. Manufacturers should provide this information to their client laboratories upon request.

Interpretive Guidelines §493.1256(c)

CONTROL PROCEDURES

In determining the control procedures, including the frequency of testing controls that detect immediate errors and monitor test performance over time, the laboratory needs to consider the following:

- *Control procedures specified by the test system's manufacturer;*
- *Test system instrument and reagent stability (e.g., relocation);*
- *Frequency and volume of test performance;*
- *Technique dependence of the method;*
- *Frequency of quality control failures; and*
- *Training, experience, and competency of technical personnel.*

Traditionally, laboratories have tested two levels of external control materials daily to monitor the accuracy and precision of the analytic test system components. External control materials have a similar matrix to that of patient specimens, are treated in the same manner as patient specimens, and go through all analytic phases of testing. External control materials may be provided as part of the test system, provided separately or prepared in-house. Testing external controls meets the requirement for monitoring test system components, environment, and operator performance. External control materials may be:

- *Commercially or in-house prepared controls;*
- *Proficiency testing specimens for which results have been confirmed;*
- *Reference or control strains of microorganisms;*
- *Calibrators of different lot numbers and concentration than those used to calibrate the system; or*
- *Previously tested patient specimens provided the laboratory determines the acceptable performance level for the patient specimens.*

D5445

§493.1256 Standard: Control procedures.

(d)Unless CMS approves a procedure, specified in Appendix C of the State Operations Manual (CMS Pub. 7), that provides equivalent quality testing, the laboratory must--

- (d)(1) Perform control procedures as defined in this section unless otherwise specified in the additional specialty and subspecialty requirements at §§493.1261 through 493.1278.
- (d)(2) For each test system, perform control procedures using the number and frequency specified by the manufacturer or established by the laboratory when they meet or exceed the requirements in paragraph (d)(3) of this section.

Interpretive Guidelines §493.1256(d)

Considerations for establishing equivalent quality testing

If the laboratory chooses to implement the reduced QC frequency for multiple instruments (including the same make and model used to perform the same test) a successful evaluation process must be performed for each instrument for which the (QC) frequency applies.

NOTE: *The regulations require laboratories to follow test system manufacturer's instruction for performing the testing. This means the laboratory must perform and follow the manufacturer's package insert as approved or cleared by the FDA.*

Advancements in laboratory technology have led to test systems that often include internal monitoring systems (electronic, internal, procedural controls, etc.). Electronic controls only monitor the electrical or electronic components of the test system. Internal or procedural controls may only monitor a portion of the analytic process, such as sample addition, instrument/reagents interaction, or test completion. These advancements may allow laboratories flexibility in determining control procedures that provide equivalent quality procedures to the traditional daily testing of two levels of external control materials. However, under no circumstances may the laboratory reduce the frequency of testing external control materials to less than that specified by the manufacturer's test system instructions.

NOTE: *Since the purpose of control testing is to detect immediate errors and monitor performance over time, increasing the interval between control testing (i.e., weekly, or monthly) will require a more extensive evaluation of patient test results when a control failure occurs (see §493.1282). The director must consider the laboratory's clinical and legal responsibility for providing accurate and reliable patient test results versus the cost implications of reducing the quality control testing frequency.*

Identifying Sources of Error

As a first step, the laboratory must determine the test system's sources of error. The test system instructions (product insert) may contain this information. If this information is not provided, the laboratory should contact the manufacturer to obtain this information in writing and include it in the procedure manual.

Test Systems with Internal and/or Procedural Controls

If internal or procedural controls are provided as part of the test system, the following information must be determined by the laboratory:

- *Whether the internal/procedural control(s) monitor all components of the test system. This information may be included in the package insert. If not, the laboratory must contact the test system's manufacturer to obtain written documentation identifying the components of the test system monitored by the internal/procedural controls and include this information in the laboratory's procedure manual;*
- *If all components are not monitored, identify those components of the test system that are monitored by the internal/procedural control(s);*
- *Have a mechanism for monitoring those components of the test system not monitored by the internal/procedural control(s); and*
- *Evaluate the affect of adverse environmental conditions and the influence of operator variance and techniques.*

NOTE: Although manufacturers may assist laboratories by providing quality control instructions, the laboratory is ultimately responsible for the performance of appropriate quality control procedures, including the documentation and interpretation of quality control data. Under subpart M, the director is responsible for ensuring that quality control (use D6020 or D6093 as appropriate) and quality assessment (use D6021 or D6094 as appropriate) programs are established and maintained to assure the quality of laboratory services, including the identification of failures in quality as they occur (use D6022 and D6094).

Equivalent Quality Control Procedures

The equivalent quality control procedures described below **may only be used** for laboratory testing subject to the following control procedure requirements:

- §493.1256(d)(3)(i-iii) – control requirements for quantitative, qualitative and semi-quantitative procedures
- §493.1256(d)(3)(iv) -- test procedures that include an extraction phase (limited to 1 and 2 below)
- §§493.1267-493.1269 – control requirements for routine chemistry and hematology (limited to 1 and 2 below)

As further technological advances are made and additional data becomes available, CMS will, as appropriate, revise the equivalent quality control procedures and/or the eligibility requirements for test procedures that may use equivalent quality control.

1. Test Systems with Internal/Procedural Control(s) that Monitor the Entire Analytic Process

If a test system uses one or more internal/procedural control(s) to monitor all of its analytic components and the laboratory using the test system successfully completes the evaluation process described below to demonstrate test system stability over time, the laboratory may use the equivalent quality control procedures described below in lieu of performing the applicable procedures specified in the regulations at §493.1256(d)(3)(i-iv) and the applicable specialty and subspecialty requirements listed for routine chemistry and hematology at §§493.1267-493.1269.

Evaluation Process: The laboratory must perform the test system's internal control procedure(s) in accordance with the manufacturer's instructions (but not less frequently than once each day of testing) and test two levels of external control material daily for 10 consecutive days of testing.

- If the internal and external control results are acceptable throughout the evaluation process, the laboratory may reduce the frequency of testing two levels of external control material from daily to **once per calendar month unless the manufacturer requires more frequent and/or additional external control testing**. The laboratory must continue to perform and monitor the internal control(s) in accordance with the manufacturer's instructions, but not less frequently than once each day of testing.
- If any internal or external control result is unacceptable during the evaluation process or after the laboratory has reduced the frequency for testing external control materials, the laboratory must repeat the unacceptable internal and/or external control.
 - If the repeat control result(s) are within range, no further corrective action is necessary and the laboratory may, as applicable, resume the evaluation process or continue the reduced frequency of external control testing.

- If the repeat control result(s) are not acceptable, the laboratory must identify the problem, take appropriate corrective action and follow the requirements at §493.1282(b)(2) before reporting patient test results. The laboratory must **restart** and successfully complete the evaluation process before reducing the frequency of testing external control materials.

- All evaluation process and corrective action activities must be documented.

Note: If a laboratory's existing QC data for the test system meets the evaluation process protocol described above, the laboratory may reduce the frequency for testing external control materials as specified above.

The laboratory must perform calibration verification (§493.1255), as applicable, and test external control materials (§493.1256) with each complete change of reagents, with each new lot number or shipment of reagents, following major preventive maintenance, or replacement of critical parts that may influence test performance. If the calibration verification and external control results are acceptable, the laboratory may continue monthly external control and daily internal control testing.

For each test, the following ongoing assessment activities are also required:

- Proficiency testing:
 - Results must demonstrate acceptable/satisfactory performance as specified in subpart H;
 - Acceptable performance must be demonstrated for testing for which proficiency testing is not required or proficiency testing materials are not available (§493.1236);
- Analytic system quality assessment (§493.1289) activities must demonstrate problems are not occurring; and
- Competency assessment evaluations must demonstrate testing personnel are accurately performing testing as specified in subpart M.

If unacceptable results are obtained for any of the above assessment activities, the laboratory must investigate, identify the problem, document the corrective action(s) taken, and **restart** the evaluation process.

2. Test Systems with Internal/Procedural Control(s) that Monitor a Portion of the Analytic Process

Some internal/procedural controls monitor only certain components of the test system. Although the test system's manufacturer may suggest other mechanisms to monitor the component(s) not checked by the internal/procedural controls, the laboratory is ultimately responsible for ensuring that all components of the analytic process are monitored. The laboratory may use the equivalent quality control procedures listed below in lieu of performing the applicable procedures specified in the regulations at §493.1256(d)(3)(i-iv) and the applicable specialty and subspecialty requirements listed for routine chemistry and hematology at §§493.1267-493.1269, when it can demonstrate the test system's stability over time. This may be substantiated by successfully completing the evaluation process (described below).

Evaluation Process: The laboratory must perform the test system's internal control procedure(s) in accordance with the manufacturer's instructions (but not less frequently than once each day of testing) and test two levels of external control material daily for 30 consecutive days of testing.

- If the internal and external control results are acceptable throughout the evaluation process, the laboratory may reduce the frequency of testing two levels of external control material from daily to once per calendar week unless the manufacturer requires more frequent and/or additional external control testing. The laboratory must continue to perform and monitor the internal control(s) in accordance with the manufacturer's instructions, but not less frequently than once each day of testing.
- If any internal or external control result is unacceptable during the evaluation process or after the laboratory has reduced the frequency for testing external control materials, the laboratory must repeat the unacceptable internal and/or external control.

- If the repeat control result(s) are within range, no further corrective action is necessary and the laboratory may, as applicable, resume the evaluation process or continue the reduced frequency of external control testing.
 - If the repeat control result(s) are not acceptable, the laboratory must identify the problem, take appropriate corrective action and follow the requirements at §493.1282(b)(2) before reporting patient test results. The laboratory must restart and successfully complete the evaluation process before reducing the frequency of testing external control materials.
- All evaluation process and corrective action activities must be documented.

Note: If a laboratory's existing QC data for the test system meets the evaluation process protocol described above, the laboratory may reduce the frequency for testing external control materials as specified above.

The laboratory must perform calibration verification (§493.1255), as applicable, and test external control materials (§493.1256) with each complete change of reagents, with each new lot number or shipment of reagents, following major preventive maintenance, or replacement of critical parts that may influence test performance. If the calibration verification and external control results are acceptable, the laboratory may continue weekly external control and daily internal control testing.

For each test, the following ongoing assessment activities are also required:

- Proficiency testing:
 - Results must demonstrate acceptable/satisfactory performance as specified in subpart H;
 - Acceptable performance must be demonstrated for testing for which proficiency testing is not required or proficiency testing materials are not available (§493.1236);
- Analytic system quality assessment (§493.1289) activities must demonstrate problems are not occurring; and
- Competency assessment evaluations must demonstrate testing personnel are accurately performing testing as specified in subpart M.

If unacceptable results are obtained for any of the above assessment activities, the laboratory must investigate, identify the problem, document the corrective action(s) taken and restart the evaluation process.

3. Test Systems without Internal/Procedural Control(s)

Test systems without internal/procedural controls subject to the extraction phase control requirements at §493.1256(d)(3)(iv) or the specialty or subspecialty requirements at §§493.1261-493.1278 are not eligible for this option.

Advancements in laboratory technology have led to the production of test systems that are capable of maintaining stable performance specifications over time and are minimally influenced by adverse environmental conditions and operator variance. While the test system manufacturer should provide the laboratory with written documentation of the test system's stability (which may be included as part of the package insert or operator manual, and must be maintained by the laboratory), the laboratory is responsible for ensuring that all components of the analytic process are monitored. This may be accomplished by testing, at a minimum, two levels of external control material daily. The laboratory may use the equivalent quality control procedures described below in lieu of performing the applicable procedures specified in the regulations at 493.1256(d)(3)(i-iii), when it can demonstrate the test system's stability over time. This may be substantiated by successfully completing the evaluation process (described below).

Evaluation Process: The laboratory must perform the test system's control procedures in accordance with the manufacturer's instructions and, at a minimum, test two levels of external control material daily for 60 consecutive days of testing. Because the test system's performance may be affected by operator variance, all personnel who will perform the test must participate in the evaluation.

- If the external control results are acceptable throughout the evaluation process, the laboratory may reduce the frequency of testing two levels of external control material from daily to **once per calendar week unless the manufacturer requires more frequent and/or additional external control testing.**
- If any external control result is unacceptable during the evaluation process or after the laboratory has reduced the frequency for testing external control material, the laboratory must repeat the unacceptable external control.
 - If the repeat control result(s) are within range, no further corrective action is necessary and the laboratory may, as applicable, resume the evaluation process or continue the reduced frequency of external control testing.
 - If the repeat control result(s) are not acceptable, the laboratory must identify the problem, take appropriate corrective action and follow the requirements at §493.1282(b)(2) before reporting patient test results. **The laboratory must restart and successfully complete the evaluation process before reducing the frequency of testing external control materials.**
- All evaluation process and corrective action activities must be documented.

Note: If a laboratory's existing QC data for the test system meets the evaluation process protocol described above, the laboratory may reduce the frequency for testing external control materials as specified above.

The laboratory must perform calibration verification (§493.1255), as applicable, and test external control materials (§493.1256) with each complete change of reagents, with each new lot number or shipment of reagents, following major preventive maintenance, or replacement of critical parts that may influence test performance. If the calibration verification and external control results are acceptable, the laboratory may continue weekly external control testing.

For each test, the following ongoing assessment activities are also required for each test:

- Proficiency testing:
 - Results must demonstrate acceptable/satisfactory performance as specified in subpart H;
 - Acceptable performance must be demonstrated for testing for which proficiency testing is not required or proficiency testing materials are not available (§493.1236);
- Analytic system quality assessment (§493.1289) activities must demonstrate problems are not occurring; and
- Competency assessment evaluations must demonstrate testing personnel are accurately performing testing as specified in subpart M.

If unacceptable results are obtained for any of the above assessment activities, the laboratory must investigate, identify the problem, document the corrective action(s) taken and **restart** the evaluation process.

§493.1256 Standard: Control procedures.

(d)(3) At least once each day patient specimens are assayed or examined perform the following for-

Interpretive Guidelines §493.1256(d)(3)

NOTE: *Throughout the analytic systems section, the regulations require laboratories to follow test system manufacturer's instruction for performing the testing. This means the laboratory must perform and follow all manufacturer's recommendations and suggestions for testing as well as those that are required to be followed.*

Laboratories must follow manufacturers' test system instructions for control performance

and meet the requirements in this section. The laboratory must determine if more extensive (e.g., number, frequency) control testing is necessary. Use D5425.

Immunology:

Determine which immunological methods the laboratory uses and how the laboratory tests quality control materials to check each test component of the test system. Examples of test systems that have multiple components are:

- Complement Fixation (CF);
- Hemagglutination inhibition (HAI);
- Radio-immunoassay (RIA);
- Enzyme immunoassay (EIA);
- Indirect immunofluorescence (IFA);
- Fluorescence Polarization Immunoassay (FPIA);
- Radioimmunoprecipitin assay (RIPA); and
- Radioallergosorbent test (RAST).

Use D5449 or D5451, as appropriate.

Syphilis Serology:

For FTA-ABS tests, does the laboratory employ:

- Reactive control serum in Phosphatase Buffered Solution (PBS);
- Reactive control serum in sorbent;
- Minimally reactive control 1+;
- Non-specific serum control in PBS;
- Non-specific serum control in sorbent;
- Non-specific staining control of PBS; and
- Non-specific staining control of sorbent?

For MHATP or HATTS tests, does the laboratory employ:

- Reactive reference control material;
- Non-reactive reference control material;
- Unsensitized erythrocyte with each specimen;
- Unsensitized erythrocyte with buffer;
- Sensitized erythrocyte with buffer;
- Unsensitized erythrocyte with each reactive control serum; and
- Unsensitized erythrocyte with non-reactive control serum?

Use D5451.

Probes §493.1256(d):

What data does the laboratory have to support its frequency of testing quality control samples?

How does a mobile laboratory evaluate instrument and reagent stability following relocation to determine the frequency of testing quality control samples?

D5447

§493.1256 Standard: Control procedures.

(d)(3)(i) Each quantitative procedure, include two control materials of different concentrations;

Interpretive Guidelines 493.1256(d)(3)(i)

For monitoring the abnormal range, the laboratory must select controls that correlate with the patient values either in terms of specimen matrix or range to be evaluated.

Routine Chemistry:

For monitoring the abnormal range, the laboratory should select control materials that correlate with the patient values both in terms of specimen matrix and range to be evaluated. For example, an elevated serum based bilirubin control should be employed when measuring neonatal bilirubins; a low level protein control or cerebrospinal fluid control should be used for monitoring cerebrospinal fluid protein.

Hematology:

For instruments which perform hemoglobin, hematocrit, red and white blood cell counts, platelets and/or differentials, acceptable controls are 2 levels of assayed materials, OR 1 level of assayed material and 1 patient specimen that was verified in the same batch of specimens with the assayed control material. The laboratory must establish criteria for an acceptable range of performance as required at D5481.

EXCEPTION:

Unless otherwise required by the test system's manufacturer or the laboratory's performance specifications, for instruments that perform white blood cell differentials directly from blood films (smears), a commercial control or patient specimen (differential) that has been verified through repetitive testing is an acceptable control and satisfies the requirements of §493.1256(d), as appropriate.

D5449

§493.1256 Standard: Control procedures.

(d)(3)(ii) Each qualitative procedure, include a negative and positive control material;

Interpretative Guidelines §493.1256(d)(3)(ii)

Urinalysis

Photomicrographs or charts of all possible urine sediment components will meet the control requirement for manual microscopic urinalysis examinations. Use D5445.

D5451

§493.1256 Standard: Control procedures.

(d)(3)(iii) Test procedures producing graded or titered results, include a negative control material and a control material with graded or titered reactivity, respectively;

Interpretive Guidelines §493.1256(d)(3)(iii)

For tests in which patient results are reported in terms of graded reactivity (1+, 2+, 3+, etc.) control(s) of graded reactivity must be used. For tests in which patient results are reported as a titer, controls of known titer must be used.

EXCEPTIONS:

A negative control is not required for anti-streptolysin O titer, anti-hyaluronidase titer tests. A positive control is not required for the cold agglutination test. For radial immuno-diffusion, one control or calibration material is required on each plate.

D5453

§493.1256 Standard: Control procedures.

(d)(3)(iv) Each test system that has an extraction phase, include two control materials, including one that is capable of detecting errors in the extraction process; and

Interpretive Guidelines §493.1256(d)(3)(iv)

Test systems with an extraction phase(s) are not eligible for the equivalent quality testing protocol.

Bacteriology:

For direct antigen systems, laboratories may use bacterial cell suspensions to meet the requirement for control organisms since the cell suspensions are subjected to both the extraction and reaction phases of the test. However, a matrix similar to patient specimens is preferred. For example, for direct antigen tests for group A streptococcal antigen, commercially prepared, dried (solid-shafted) swabs, one containing group A streptococcus (*S. pyogenes*) as a positive control and another with non-group A streptococcus and/or *Staphylococcus aureus* as a negative control may be used.

Additionally, if the manufacturer's instructions do not specify what the positive control contains, the laboratory should contact the manufacturer to ensure that the positive control contains a cell suspension of the organism. Otherwise, the laboratory must have an alternative mechanism for meeting this requirement (e.g., laboratory suspension stock ATCC organism, commercially prepared organism controls).

Toxicology:

For gas chromatography and mass spectrometry used for drug confirmations, an analyte specific control is required for both qualitative and quantitative tests.

For comprehensive broad spectrum qualitative drug screening procedures using gas chromatography, a control material containing one or more drugs representative of each drug class reported [e.g., tricyclic antidepressants, barbiturates] must go through each test phase, including the extraction process.

D5455

§493.1256 Standard: Control procedures.

(d)(3)(v) Each molecular amplification procedure, include two control materials and, if reaction inhibition is a significant source of false negative results, a control material capable of detecting the inhibition.

Interpretive Guidelines §493.1256(d)(3)(iii)

The laboratory is also responsible for following the manufacturer's instructions concerning procedure limitations for detecting nucleic acid target amplification sequences, when provided by the manufacturer.

If the laboratory suspects the presence of interfering substances (inhibitors), the laboratory is responsible for using a control material (in addition to positive and negative control materials) capable of detecting interfering substances. Patient specimens may contain substances (inhibitors) that interfere with the enzymatic reaction of a molecular amplification procedure. These interfering substances could affect the assay's sensitivity causing a false negative result. Interfering substances may include, but are not limited to components within the patient specimen or exogenous substances introduced during the preanalytic and/or analytic phase of testing.

D5457

§493.1256 Standard: Control procedures.

(d)(4) For thin layer chromatography-

(i) Spot each plate or card, as applicable, with a calibrator containing all known substances or drug groups, as appropriate, which are identified by thin layer chromatography and reported by the laboratory; and

(d)(4)(ii) Include at least one control material on each plate or card, as applicable, which must be processed through each step of patient testing, including extraction processes.

Interpretive Guidelines §493.1256(d)(4)

For qualitative urine drug screens performed by thin layer chromatography, a negative control is not required. However, a control containing one or more drugs representative of each drug group reported (e.g., tricyclic antidepressants, barbiturates) that goes through each test phase (including the extraction process) is required.

D5459

§493.1256 Standard: Control procedures.

(d)(5) For each electrophoretic procedure include, concurrent with patient specimens, at least one control material containing the substances being identified or measured.

D5461

§493.1256 Standard: Control procedures.

(d)(6) Perform control material testing as specified in this paragraph before resuming patient testing when a complete change of reagents is introduced; major preventive maintenance is performed; or any critical part that may influence test performance is replaced.

D5463

§493.1256 Standard: Control procedures.

(d)(7) Over time, rotate control material testing among all operators who perform the test.

Interpretive Guidelines §493.1256(d)(7)

The laboratory may use this requirement to assist in competency assessment determinations specified in subpart M.

D5465

§493.1256 Standard: Control procedures.

(d)(8) Test control materials in the same manner as patient specimens.

Interpretive Guidelines §493.1256(d)(8)

Control materials of a similar matrix to that of patient specimens should be utilized, if available, and the control materials must be treated in the same manner as patient specimens and go through all analytic test phases.

Flow Cytometry

In cell surface phenotyping by flow cytometry or fluorescent microscopy, control

samples must be analyzed within the same time period after staining as test specimens.

Probes §493.1256(d)(8)

Flow Cytometry

How did the laboratory establish the time period in which stained cells must be analyzed to avoid significant loss of any cell subpopulations or total cell numbers?

If analysis will be based on a population of cells selected by flow cytometry "gating" on size or density parameters, or selected by depletion or enrichment techniques, are controls tested with each patient to detect the presence of contaminating cells in the selected population? (e.g., Monocyte contamination of "lymphocytes" gated by forward angle or forward angle versus 90° light scatter must be detected with a monocyte-specific antibody.) Use D5465 or D5425 as appropriate.

D5467

§493.1256 Standard: Control procedures.

(d)(9) When using calibration material as a control material, use calibration material from a different lot number than that used to establish a cut-off value or to calibrate the test system.

D5469

§493.1256 Standard: Control procedures.

(d)(10) Establish or verify the criteria for acceptability of all control materials.

(d)(10)(i) When control materials providing quantitative results are used, statistical parameters (for example, mean and standard deviation) for each batch and lot number of control materials must be defined and available.

(d)(10)(ii) The laboratory may use the stated value of a commercially assayed control material provided the stated value is for the methodology and instrumentation employed by the laboratory and is verified by the laboratory.

(d)(10)(iii) Statistical parameters for unassayed control materials must be established over time by the laboratory through concurrent testing of control materials having previously determined statistical parameters.

Interpretive Guidelines §493.1256(d)(10)

Acceptable ranges must be verified (assayed) or established (unassayed) by the laboratory for control materials and any calibrators that are used in lieu of control materials.

For procedures in which a spiked sample is used as a control, an acceptable range must be established for the amount of recovery of the spiked sample, either in percentage or actual concentration.

If laboratories rely on commercial companies to establish statistical limits for controls, the laboratory must have documentation to verify that its control results correlate with the established limits.

When patient specimens are used to meet the control requirements, data must be evaluated in accordance with §493.1256(d)(10)(iii).

There are no specific guidelines for the number of times a material must be tested to

establish statistical limits. In general, twenty replicate tests should be considered the minimum for determining a standard deviation.

Probes §493.1256(d)(10)

What statistics does the laboratory have to demonstrate the number of assays and the period of time in which the laboratory repetitively tested control materials to verify or establish control limits?

How does the laboratory evaluate control results to detect any outliers, shifts or trends in control values due to instrument malfunctions or changes in the analytical system?

If more than one test system is in use for a test procedure, did the laboratory evaluate the data for each test method in the establishment of control limits?

§493.1256 Standard: Control procedures.

(e) For reagent, media, and supply checks, the laboratory must do the following:

D5471

§493.1256 Standard: Control procedures.

(e)(1) Check each batch (prepared in-house), lot number (commercially prepared) and shipment of reagents, disks, stains, antisera, (except those specifically referenced in §493.1261 (a)(3)) and identification systems (systems using two or more substrates or two or more reagents, or a combination) when prepared or opened for positive and negative reactivity, as well as graded reactivity, if applicable.

Interpretive Guidelines §493.1256(e)(1)

Review the laboratory's quality control records and note when lot numbers change.

NOTE: Media checks are defined under §493.1256(e)(4) Guidelines.

The laboratory must demonstrate that each reagent performs within the specifications established by the laboratory for the test procedure. Documentation of concurrent testing of reagents or acceptable quality control results will satisfy this requirement.

Reagents, disks, and test procedures used for identification purposes may include, but are not limited to, catalase, coagulase plasma, oxidase, bacitracin, optochin, CefinaseTM, ONPG, X and V factor strips and disks, germ tube, yeast morphology media, and commercial identification systems.

A negative reactivity control is not required for the mycology germ tube test.

Test each batch, lot, and shipment for positive and negative reactivity for reagents such as:

- *Bacitracin;*
- *Catalase;*
- *Cefinase;*
- *Coagulase plasma;*
- *ONPG;*
- *Optochin;*

- Oxidase;
- Spot indole; and
- X and V factor strips and disks.

For bacteriology, XV discs or strips need only be checked with an organism that produces a positive reaction.

Probes §493.1256(e)(1)

What records does the laboratory have to demonstrate that controls are tested when shipments of reagents, discs, stains, antisera or identification systems are opened or when the laboratory prepares these materials? Use D5471 for not recording performance and for nonperformance of quality control checks, and stain checks.

D5473

§493.1256 Standard: Control procedures.

(e)(2) Each day of use (unless otherwise specified in this subpart), test staining materials for intended reactivity to ensure predictable staining characteristics. Control materials for both positive and negative reactivity must be included, as appropriate.

Interpretive Guidelines §493.1256(e)(2)-(e)(3)

Acid-fast stains must be checked each day of use for positive and negative reactivity.

D5475

§493.1256 Standard: Control procedures.

(e)(3) Check fluorescent and immunohistochemical stains for positive and negative reactivity each time of use.

Interpretive Guidelines §493.1256(e)(3)

All fluorescent stains, including fluorochrome acid-fast stains, must be tested for positive and negative reactivity each time of use.

Flow Cytometry

Staining controls for cell surface immunophenotyping by flow cytometry should consist of either normal, cultured or abnormal cells known to be positive for selected standard antigens and must verify the proper performance of reagents. Frozen or other preserved cells may be used. A negative reagent control must be run for each test cell preparation, and is to consist of monoclonal antibody(ies) of the same species and isotype. Negative reagent controls will consist of:

- *For indirect stains, an irrelevant primary antibody, if available, and in all cases, the same secondary antibody(ies) conjugated with the same fluorochrome(s) used in all relevant test combinations; and*
- *For direct stains, an irrelevant antibody conjugated to the same fluorochrome and at the same fluorochromes:protein ratio used in all relevant test combinations.*

Probes §493.1256(e)(3)

For flow cell cytometric surface immunophenotyping, is a negative reagent control used to define a threshold for positive staining cells? If not, how does the laboratory define the threshold for positive staining cells?

D5477

§493.1256 Standard: Control procedures.

(e)(4) Before, or concurrent with the initial use-

Check each batch of media for sterility if sterility is required for testing;

(e)(4)(ii) Check each batch of media for its ability to support growth and, as appropriate, select or inhibit specific organisms or produce a biochemical response; and

(e)(4)(iii) Document the physical characteristics of the media when compromised and report any deterioration in the media to the manufacturer.

Interpretive Guidelines §493.1256(e)(4)

A batch of media (solid, semi-solid, or liquid) consists of all tubes, plates, or containers of the same medium prepared at the same time and in the same laboratory; or, if received from an outside source or commercial supplier, consists of all of the plates, tubes or containers of the same medium that have the same lot numbers and are received in a single shipment.

A sample from each batch of media is sufficient as a check for:

- *Sterility, if it is autoclaved or filtered during preparation;*
- *Ability to support growth, using at least one organism to demonstrate the ability of the media to support growth;*
- *Selectivity and/or inhibition, using at least one organism to confirm its selective characteristic, and at least one organism to confirm its inhibitory characteristic; and*
- *Biochemical response, using at least one organism which will produce the expected reaction (positive control) and with at least one organism which will not produce the expected reaction (negative control).*

EXCEPTION:

*A laboratory using commercially prepared microbiological culture media that is quality controlled in accordance with the NCCLS Approved Standard (M22-A2) Table 2*** need not perform quality control checks for sterility, growth, selectivity and/or inhibition and biochemical responses provided:*

- *The laboratory has documentation on the media label or brochure that the quality control practices conform to NCCLS specifications; and*
- *The laboratory documents receipt and condition of each batch of media, and notifies the media manufacturer of:*
 - *Cracked petri dishes;*
 - *Unequal filling of plates;*
 - *Cracked media in plates;*
 - *Hemolysis;*
 - *Freezing;*
 - *Excessive number of bubbles; and*
 - *Contamination.*
 -

This exception does not apply to:

- *Campylobacter agar;*
- *Chocolate agar;*
- *Media for the selective isolation of pathogenic Neisseria;*
- *Other media not listed on Table 2 (e.g., dermatophyte test medium);*
- *Media used for the isolation of parasites, viruses, mycoplasmas, chlamydia;*
- *Mueller-Hinton media used for antimicrobial susceptibility tests; or*

- *Media commercially prepared and packaged as a unit or system consisting of two or more different substrates, primarily used for microbial identification.*

Although Campylobacter medium, chocolate agar and media for the selective isolation of pathogenic Neisseria are listed on Table 2, these media must be retested by the user laboratory because these media have demonstrated higher failure rates.

American Type Culture Collection (ATCC) control organisms are not necessarily required. However, if the laboratory uses "in-house" isolates for control organisms, it must have established reactivity for each organism. Use D5469.

*Central laboratories that prepare media for satellite locations must either perform the same quality control checks required of commercial manufacturers listed on Table 2*** of the NCCLS Approved Standard (M22-A2) and furnish documentation of media quality control checks to each satellite location, or each laboratory must continue to perform media checks as required under §493.1256(e)(1).*

If a laboratory screens cultures for growth or no growth, reports "No growth" and refers all growth to a reference laboratory, the screening laboratory must perform applicable quality control of the media.

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Table 2. Manufacturers' Quality Assurance Procedure for Commercially Prepared Media

Medium	Atmosphere, Length of Incubation ¹	Control Organisms (ATCC No.) ²	Expected Results
Anaerobic sheep blood and laked blood agar media (non-selective)	Anaerobic, 24–48 h	<i>B. fragilis</i> (25285) <i>C. perfringens</i> (13124) <i>F. nucleatum</i> (25586) <i>P. anaerobius</i> (27337)	Growth Growth, beta hemolysis Growth Growth
Anaerobic broths - see Thioglycollate medium			
Blood Agar-nonselective sheep blood agar media	Aerobic or CO ₂ , 24 h	<i>S. pyogenes</i> (19615) <i>S. pneumoniae</i> (6305) <i>S. aureus</i> (25923) <i>E. coli</i> (25922)	Growth, beta hemolysis Growth, alpha hemolysis Growth Growth
Blood Agar-CAMP Test (TSA with sheep blood only)	Aerobic, 24 h	<i>S. aureus</i> (33862) or <i>S. aureus</i> (25923) <i>S. agalactiae</i> (12386) <i>S. pyogenes</i> (19615)	Positive reaction (arrowhead area of clearing) Negative reaction (no arrowhead formation)
Blood Agar-Selective sheep blood agar media (Columbia CNA Agar, phenylethyl alcohol agar)	Columbia CNA CO ₂ , 24–48h	<i>S. pyogenes</i> (19615) <i>S. pneumoniae</i> (6305) <i>S. aureus</i> (25923) <i>P. mirabilis</i> (12453)	Growth, beta hemolysis Growth, alpha hemolysis Growth Inhibition (partial)
	Phenylethyl alcohol agar CO ₂ , 24–48h	<i>S. pyogenes</i> (19615) <i>S. aureus</i> (25923) <i>P. mirabilis</i> (12453)	Growth Growth Inhibition (partial)
Blood culture media (This applies to BHI, TSB, and Thiol-based media. Other media for blood culture are exempt from user performance testing provided that manufacturers certify that additional organisms appropriate for their intended use have been tested.)	Anaerobic (nonvented) within 5 days	<i>B. fragilis</i> (25285) <i>S. pneumoniae</i> (6305)	Growth Growth
	Aerobic (vented) within 5 days	<i>P. aeruginosa</i> (27853) <i>S. pneumoniae</i> (6305)	Growth Growth
Campylobacter agar (User quality control required.)	Reduced O ₂ , enriched with CO ₂ , 42 °C, 48 h	<i>C. jejuni</i> (33291) <i>E. coli</i> (25922)	Growth Inhibition (partial)

Table 2. Manufacturers' Quality Assurance Procedure for Commercially Prepared Media (Continued)

Medium	Atmosphere, Length of Incubation ¹	Control Organisms (ATCC No.) ²	Expected Results
Chocolate agar	CO ₂ , 24 and 48 h	<i>N. gonorrhoeae</i> (43069 or 43070) <i>H. influenzae</i> (10211)	Growth Growth
CIN Agar	Aerobic, 24–48 h 25 °C	<i>Y. enterocolitica</i> (9610) <i>E. coli</i> (25922) <i>P. aeruginosa</i> (27853) <i>E. faecalis</i> (29212)	Growth; deep red center, transparent border (bulls eye) Inhibition (partial to complete) Inhibition (partial to complete) Inhibition (partial to complete)
CLED Agar	Aerobic, 24–48 h	<i>E. coli</i> (25922) <i>P. vulgaris</i> (8427) <i>S. aureus</i> (25923)	Growth; yellow centers Growth; bluish, spreading inhibited (partial) Growth; uniform deep yellow
CYE/BCYE Agar	Aerobic, 48–72 h	<i>L. pneumophila</i> (33152) <i>L. bozemanii</i> (33217)	Growth; yellow-green fluorescence under long-wave u.v. light Growth; blue-white fluorescence under long-wave u.v. light
Enrichment broths for enterics (GN Broth, Selenite Broths)	Aerobic, up to 24 h	<i>S. typhimurium</i> (14028) <i>S. sonnei</i> (9290) <i>E. coli</i> (25922)	Growth on subculture Growth on subculture (may be inhibited by Selenite media) Inhibition (partial to complete) on subculture. Growth on subculture from GN broth
Eosin methylene blue media (Levine EMB Agar; EMB Agar, modified)	Aerobic, 24 h	<i>S. typhimurium</i> (14028) <i>E. coli</i> (25922) <i>E. faecalis</i> (29212)	Growth, colorless to amber colonies Growth, blue-black colonies w/green metallic sheen Inhibition (partial)
Hektoen enteric agar	Aerobic, 24 h	<i>S. typhimurium</i> (14028) <i>S. flexneri</i> (12022) <i>E. faecalis</i> (29212) <i>E. coli</i> (25922)	Growth, colonies blue to green-blue with black centers Growth, colonies green to blue-green Inhibition (partial; colonies yellow) Inhibition (partial to complete; colonies yellow to salmon colored)
MacConkey agar	Aerobic, 24 h	<i>E. coli</i> (25922) <i>P. mirabilis</i> (12453) <i>S. typhimurium</i> (14028) <i>E. faecalis</i> (29212)	Growth, pink colonies Growth, colorless colonies, inhibition of swarming (partial) Growth, colorless colonies Inhibition (partial)
Mannitol salt agar	Aerobic, 24 and 48 h	<i>S. aureus</i> (25923) <i>S. epidermidis</i> (12228) <i>P. mirabilis</i> (12453)	Growth, colonies have yellow zones at 48 h Growth, colonies have red zones at 48 h Inhibition (partial)

Table 2. Manufacturers' Quality Assurance Procedure for Commercially Prepared Media (Continued)

Medium	Atmosphere, Length of Incubation ¹	Control Organisms (ATCC No.) ²	Expected Results
Mycobacteria agar media (Lowenstein–Jensen and Middlebrook)	CO ₂ up to 21 days	<i>M. tuberculosis</i> H37Ra (25177) <i>M. kansasii</i> Group I (12478) <i>M. scrofulaceum</i> Group II (19981) <i>M. intracellulare</i> Group III (13950) <i>M. fortuitum</i> Group IV (6841) <i>E. coli</i> (25922)	Growth Growth Growth—May be inhibited on selective L-J and selective Middlebrook media Growth—May be inhibited on selective L-J and selective Middlebrook media Growth Inhibition (partial to complete)—Use only for selective mycobacteria media
Sabouraud dextrose agar	Aerobic, up to 7 days 25–35 °C	<i>C. albicans</i> (60193 or 10231) <i>T. mentagrophytes</i> (9533)	Growth Growth
Salmonella–Shigella (SS) agar	Aerobic, 24 h	<i>S. typhimurium</i> (14028) <i>S. flexneri</i> (12022) <i>E. faecalis</i> (29212) <i>E. coli</i> (25922)	Growth, colonies colorless with or without black centers Growth, colorless colonies Inhibition (complete) Inhibition (partial to complete; colonies pink to rose-red with precipitate)
Selective mycology media (media containing cycloheximide and chloramphenicol, excluding inhibitory mold agar)	Aerobic, up to 7 days 25 °C	<i>A. niger</i> (16404) <i>C. albicans</i> (10231) <i>T. mentagrophytes</i> (9533) <i>E. coli</i> (25922)	Inhibition (partial to complete) on media containing cycloheximide Growth Growth Inhibition (partial to complete) on media containing chloramphenicol
Selective media for pathogenic <i>Neisseria</i> spp. (<i>User quality control required.</i>)	CO ₂ , 24–48 h	<i>N. gonorrhoeae</i> (43069 or 43070) <i>N. meningitidis</i> (13090) ³ <i>P. mirabilis</i> (43071) <i>E. coli</i> (25922) ³ <i>N. sicca</i> (9913) ³ <i>C. albicans</i> (60193) ³ <i>S. epidermidis</i> (12228)	Growth Growth Inhibition (partial)—use only for media containing trimethoprim Inhibition (partial) Inhibition (partial) Inhibition (partial) Inhibition (partial)
Selective media for enterococci, with azide	Aerobic, 24 and 48 h	<i>E. faecalis</i> (29212) <i>S. pyogenes</i> (19615) <i>E. coli</i> (25922)	Growth, blackening around colonies Inhibition (partial to complete) Inhibition (partial)—Colorless colonies on bile esculin agar
Selective media for enterococci, without azide	Aerobic, 24 and 48 h	<i>E. faecalis</i> (29212) <i>S. pyogenes</i> (19615)	Growth, blackening around colonies Inhibition (partial to complete)
Thioglycolate medium, with or without indicator	Aerobic, 48 h (tightened cap)	<i>B. fragilis</i> (25285) <i>S. aureus</i> (25923)	Growth Growth
Thioglycolate medium, enriched with vitamin K and hemin	Aerobic, 48 h (tightened cap)	<i>P. anaerobius</i> (27337) <i>B. vulgatus</i> (8482) <i>C. perfringens</i> (13124)	Growth Growth Growth

Table 2. Manufacturers' Quality Assurance Procedure for Commercially Prepared Media (Continued)

Medium	Atmosphere, Length of Incubation ¹	Control Organisms (ATCC No.) ²	Expected Results
Tubed media (BHI and Tryptic Soy Broth)	Aerobic, 24-48 h	<i>E. coli</i> (25922) <i>S. aureus</i> (25923)	Growth Growth
XLD (xylose lysine desoxycholate) Agar	Aerobic, 24 h	<i>S. typhimurium</i> (14028) <i>S. flexneri</i> (12022) <i>E. faecalis</i> (29212) <i>E. coli</i> (25922)	Growth—Colonies red with black centers Growth—Colonies red Inhibition (partial) Inhibition (partial to complete; colonies yellow to yellow-red)

¹Temperature is 35 °C unless otherwise specified.
²ATCC is a registered trademark of the American Type Culture Collection.
³Required for commercial manufacturers; not necessary for testing by users.

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§493.1256 Standard: Control procedures.

(e)(5) Follow the manufacturer's specifications for using reagents, media, and supplies and be responsible for results.

NOTE: Throughout the analytic systems section, the regulations require laboratories to follow test system manufacturer's instruction for performing the testing. This means the laboratory must perform and follow all manufacturer's recommendations and suggestions for testing as well as those that are required to be followed.

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§493.1256 Standard: Control procedures.

(f) Results of control materials must meet the laboratory's and, as applicable, the manufacturer's test system criteria for acceptability before reporting patient test results.

§493.1256 Standard: Control procedures.

(g) The laboratory must document all control procedures performed.

Interpretive Guidelines §493.1256(g)

The actual measurement(s) taken, reactions and/or observations must be recorded.

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§493.1256 Standard: Control procedures.

(h) If control materials are not available, the laboratory must have an alternative mechanism to detect immediate errors and monitor test system performance over time. The performance of alternative control procedures must be documented.

Interpretive Guidelines §493.1256(h)

Laboratories may choose to split samples for testing by another method or in another laboratory to evaluate the results obtained. Previously tested patient specimens (include specimens across the reportable range) tested in duplicate. Precision is determined through replicate testing of a previously tested patient specimen. The duplicate tests may be performed by the same individual or by different people and the results compared to previously defined acceptable limits for differences between duplicates.

PUBLIC HEALTH LABORATORIES PERFORMING NEWLY DEVELOPED ASSAYS/TEST SYSTEMS FOR AGENTS FOR EMERGENT PUBLIC HEALTH SIGNIFIANCE Screening and confirmation methods for agents of emergent public health significance require the rapid development and transfer of technology and expertise from federal agencies to public health laboratories (or other designee laboratories). Because of unique situations of emergent diseases or other public health threats, control and calibration materials for the assay or test system may not be immediately available. Under these circumstances, the laboratory must follow the assay or test system's protocol(s) without modification and document the alternative mechanisms employed to

ensure accurate test results. Laboratories are encouraged to use multiple mechanisms (as described below) for ensuring accuracy.

When control and calibration materials are not available, examples of alternative control procedures that may be available include, but not limited to, the following:

- *Split specimens for testing by another method or in another laboratory;*
- *Include previously tested patient specimens (both positive and negative) tested in duplicate as surrogate controls;*
- *Test each patient specimen in duplicate;*
- *Test multiple specimen types from the same patient (e.g., saliva, urine, serum);*
- *Perform serial dilutions of positive specimens to confirm positive reactions;*
- *Provide additional supervisory review of results prior to release.*

As soon as control and calibration materials become available, the applicable requirements in §493.1256 must be met.

For specific information regarding testing for agents of emergent public health significance and alternative methods/procedures for ensuring accuracy of this testing, refer to www.aphl.org.

Probes §493.1256(h)

If control materials are not provided by the manufacturer, how does the laboratory assure the validity of test results?